

Impact of three global change drivers on a Mediterranean shrub

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Abstract. Global change is not restricted to climate change, and plant species generally face multiple human-driven disturbances constraining their viability. Most importantly, interactions among these drivers frequently generate nonadditive effects that cannot be predicted based on single-factor studies. Our goal was to assess the joint effects of three global change drivers that are especially relevant in Mediterranean ecosystems, namely, fragmentation, reduced habitat quality, and climate change on *Centaurea hyssopifolia*, a gypsum specialist plant. We carried out a two-year study (2005–2006) in natural populations of this plant in large (>11 ha) and small (<1.5 ha) fragments. Within each fragment, we identified areas of contrasting habitat quality as revealed by plant cover and nutrient content, and within each combination of habitat quality and fragment size we performed a rainfall manipulation experiment simulating the most likely future climate scenario for the region. Survival, growth, phenology, and reproductive success of selected plants were monitored. The three drivers profoundly affected responses of *Centaurea hyssopifolia* in both study years, phenology being mainly affected by changes in habitat quality and reductions in rainfall and reproductive traits being mainly affected by fragmentation. Plants in sites of poor habitat quality and plants in the dry treatment advanced most of their phenophases (flowering and dispersing earlier) and showed reduced growth rate and increased fraction of senescent leaves. Plants growing in small fragments had lower survival, lower number of viable seeds, and a reduced seed set compared to those from large fragments. We found significant synergistic interactions among drivers. For example, the interaction between fragmentation and habitat quality led to lower survival and lower relative growth in plants from small and poor-quality habitat sites. Our results highlight the importance of studies addressing simultaneously all relevant drivers of global change potentially affecting plant performance under natural conditions. In addition, the complex responses of phenology and reproductive traits of *C. hyssopifolia* emphasize the need for studies integrating traits from vegetative to reproductive and from the organ to the whole-plant level.

Key words: *Centaurea hyssopifolia*; changes in habitat quality; climate change; fitness; fragmentation; global change; gypsumophile; interactions; Mediterranean ecosystem; phenology.

INTRODUCTION

Most research on the effects of global change on plant species have focused on isolated drivers, typically on climate warming (Bakkenes et al. 2002, Badeck et al. 2004, Valladares 2008), an approach that overly simplifies realistic species' responses as discussed by Matesanz et al. (2009). Despite the growing concern for climate change impacts, global change is not restricted to climate since many drivers exert even more dramatic pressures on the ecosystems. Plant species usually face multiple, human-driven problems (e.g., land use changes and biotic invasions; Sala et al. 2000), and most importantly, interactions among these drivers frequently generate nonadditive effects that cannot be predicted based on single-factor studies (Sala et al. 2000). This becomes

especially relevant for dry Mediterranean ecosystems, which have suffered profound human-mediated transformations over centuries (UNESCO 1962, Kosmas et al. 2002). These transformations may either exacerbate or attenuate species' responses to climate change (Honnay et al. 2002, Mora et al. 2007). Recent attempts to explore the effects of concurrent global changes have generally been carried out in temperate or alpine plant communities (e.g., Honnay et al. 2002, Shaw et al. 2002), while information on other ecosystems remains scarce.

In Mediterranean environments, water is considered the primary limiting factor for plant growth and reproduction (Monteith and Webb 1981), and it is likely to become even more limiting under the increasing aridity predicted for this region (Christensen et al. 2007). This will be especially critical in semiarid gypsum environments (Escudero et al. 1999, 2000), which constitute one of the most threatened habitats in the Mediterranean Basin (European Community Council Directive 92/43/EEC 1992). Reductions in rainfall may impact plant species not only through direct

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effects on phenology or reproduction (Peñuelas et al. 2004) but also through other processes, such as disruptions of plant–plant or plant–animal interactions (Brooker 2006). Relevant studies on the impact of altered precipitation are much needed since most studies linking phenology with climate changes have focused on the effects of warming rather than on changes in rainfall (Hughes 2000, Peñuelas and Filella 2001, Walther 2004), despite the potentially stronger impact of the latter in the Mediterranean region (Christensen et al. 2007).

Most Mediterranean ecosystems have historically suffered strong fragmentation mainly due to agricultural practices, which transform the landscape into patches of natural vegetation of different sizes interspersed with dry-farmed crops (Pueyo et al. 2008). Habitat fragmentation is considered a main driver of global change and an important threat to biodiversity (Young et al. 1996). Fragmentation not only decreases population size and increases isolation, but also introduces changes at the landscape level (e.g., changes in vegetation structure, edge effects, and so on; Saunders et al. 1991). As a consequence, plant populations in fragmented landscapes may be exposed to increased inbreeding, lower individual fitness, and lower genetic variability (Ellstrand and Elam 1993, Aizen et al. 2002, Aguilar et al. 2006). Furthermore, fragmentation is usually accompanied by changes in habitat quality, i.e., soil conditions that may influence growth and performance of plants (Lindenmayer and Fischer 2006). Consequently, the impacts of reduced habitat quality have typically been considered as a concomitant result of habitat fragmentation and have been rarely studied as an individual driver of global change (Harrison and Bruna 1999, Schleuning et al. 2008). However, in agricultural landscapes, changes in habitat quality may occur beyond those promoted by the loss of habitat per se, due, for instance, to the runoff and drift of fertilizers and chemicals into the adjacent areas or to intense plowing and trampling (Boutin and Jobin 1998, Liira et al. 2008). Hence, as habitat quality may change independently from fragmentation, the impacts of a reduced habitat quality must also be specifically addressed. Impacts of habitat quality on plants are poorly understood because most studies assessing the effects of habitat quality have been carried out on animal populations, with scant information for plant populations (Lindenmayer and Fischer 2006).

The main goal of our study was to assess the joint effects of three relevant global change drivers, namely habitat fragmentation, changes in habitat quality, and water availability (climate change via reduced rainfall), on survival, phenology, growth, and reproduction of *Centaurea hyssopifolia* Vahl., a gypsum specialist shrub from central Spain. To address this objective, we performed a two-year field experiment testing the following hypotheses: (1) habitat fragmentation, changes in habitat quality, and reductions in rainfall will exert a negative effect on the performance of *Centaurea hyssopifolia*; (2) synergistic interactions among drivers will

amplify the impact on plant performance, as suggested by interactive effects of global change observed in plant species from other latitudes (e.g., Honnay et al. 2002, Shaw et al. 2002); and (3) responses will be trait-dependent. For example, phenology can be expected to be more susceptible to changes in rainfall and habitat quality (Sandvik and Totland 2000, Peñuelas et al. 2004), and reproductive traits may be more affected by fragmentation due to the alterations of plant–pollination interactions and to pernicious genetic effects on small fragments (Ellstrand and Elam 1993, Aizen et al. 2002, Aguilar et al. 2006).

METHODS

Species and study site

Centaurea hyssopifolia Vahl. (Compositae) is a cushion-like shrub endemic to semiarid central Spain. It is a gypsophile, i.e., a species that occurs only on gypsum soils. Flowering individuals produce 2–550 capitula consisting mainly of many purplish disc florets and few ray florets. Plant size ranges from 10 to 50 cm in height and from 5 to 65 cm in diameter. Flowering spans from mid-May to mid-July, and flowering peak occurs in mid-June. It is a self-incompatible species with generalist entomophilous pollination (Luzuriaga et al. 2006).

The study was carried out in a gypsum area located close to Belinchón, Spain (754 m above sea level; 75 km south from Madrid, 40°03' N, 3°03' W). In this landscape, gypsum outcrops with natural vegetation are interspersed with dry-farm crops. Vegetation is dominated by creeping and cushion chamaephytes, including a high proportion of gypsophiles, such as *Helianthemum squamatum* (L.) Dum. Cours (Cistaceae) and *Lepidium subulatum* L. (Cruciferae), and narrowly distributed endemic species, such as *Thymus lacaitae* Pau (Labiatae) and *Teucrium pumilum* L. (Labiatae). Plant cover is usually low (<30%), and bare soil areas give shelter to a conspicuous biological soil crust, formed by lichens, mosses, and cyanobacteria, most of them specialists of these soils (Martínez et al. 2006).

The area has a Mediterranean semiarid climate, with a mean annual rainfall of 433 mm, a pronounced summer drought, and a mean annual temperature of 13.8°C (56- and 21-year climatic data for rainfall and temperature, respectively; Huelves Meteorological Station, 15 km from the study site). The study was conducted over two years: 2005, which was the second driest year of the 56-year series (298 mm annual rainfall), and 2006, also a drier-than-average year, with annual rainfall of 371 mm (see Appendix A for detailed climatic data).

Experimental design

We performed a two-year field experiment with three controlled experimental factors: fragmentation, habitat quality, and water availability. The design of the experiment is composed of two levels of fragmentation, large fragments (L) and small fragments (S); two levels of habitat quality, high (H) and poor (P); and two levels

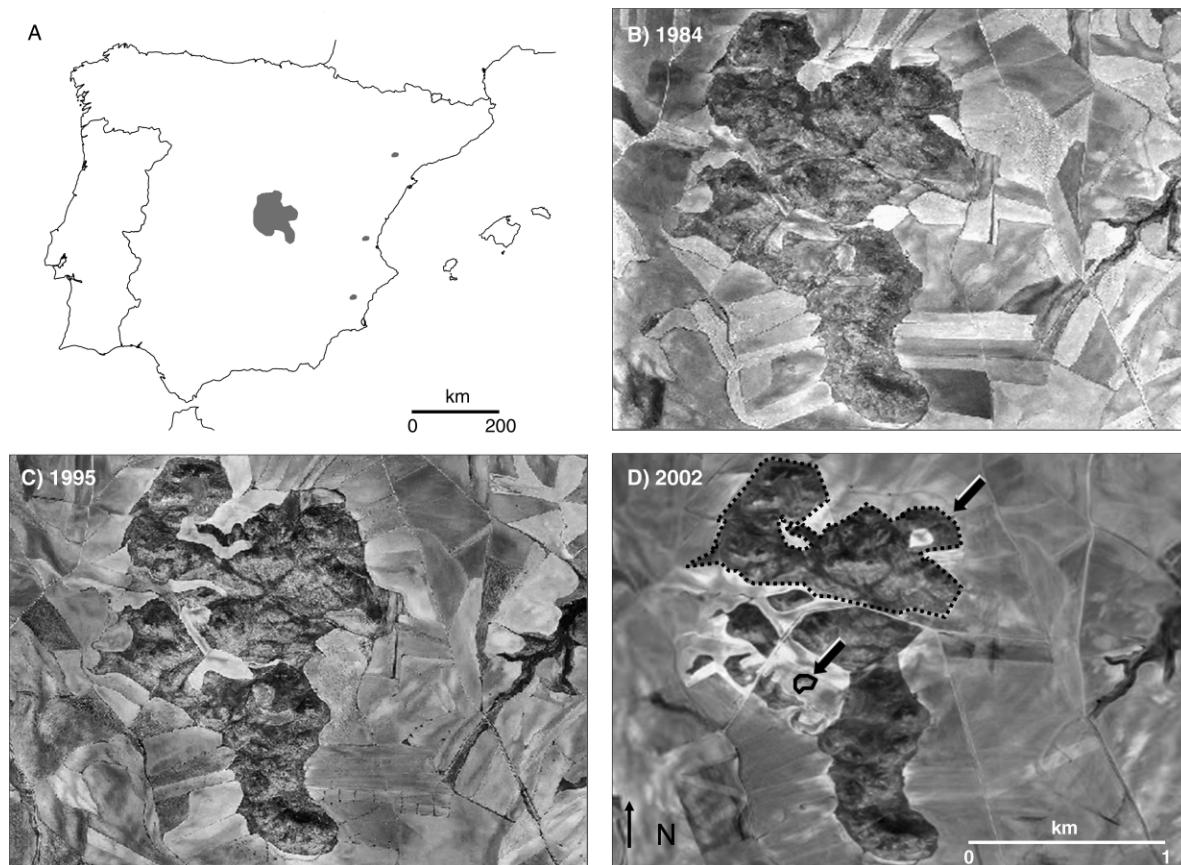


FIG. 1. (A) Distribution of *Centaurea hyssopifolia* in the Iberian Peninsula (gray area). (B–D) Fragmentation in the study site in (B) 1984, (C) 1995, and (D) 2002. The solid and dashed lines show limits of a small and a large fragment, respectively. See *Methods* for details on fragment sizes.

of water availability, mesic (M, watered plants) and dry (D, nonwatered plants).

To select the two levels of fragmentation in the field, we thoroughly surveyed the existing patches of natural vegetation islands, both in the field and by exploring the aerial photographs from the last two decades. Agriculture intensification has fragmented the landscape into scattered patches of natural vegetation of different sizes (see Fig. 1). We identified two contrasting situations: large fragments (area > 11 ha) and small fragments (area < 1.5 ha). Among them, six fragments, of which three were large fragments and three small fragments, were selected, and their limits marked with a global positioning system (GPS; Garmin II Plus, Garmin, Missouri, USA). Coordinates were exported to a global information system (GIS) software (Global Mapper Software, Parker, Colorado, USA) to calculate size and area of each fragment using the most recent aerial photograph of the study site (2002). Areas of the selected large and small fragments were 11.82, 12.34, and 37.92 ha and 0.32, 0.34, and 1.16 ha, respectively.

To define the two levels of habitat quality, areas of contrasting plant cover were randomly selected within each fragment. Habitat quality was taken as habitat

suitability, i.e., soil conditions that may improve growth and performance of plants (Lindenmayer and Fischer 2006). According to this, plant cover, as a correlative of biomass and productivity, was used as an integrative indicator of soil conditions (Dullinger et al. 2007), which were further quantified by measuring several soil and vegetation attributes (Table 1). First, percentage of bare soil, cover of perennial species, cover of annual species, cover of lichen and moss species, and cover of litter were measured in 40 subplots (1 m diameter) located within high-cover areas and 40 subplots within the low-cover ones of each fragment (480 subplots total). Second, five soil samples were collected in each high- and low-cover area (60 samples total), collecting four subsamples with a $6 \times 6 \times 10$ cm metal core, which were thoroughly mixed afterwards. Once in the laboratory, soil was sieved (2 mm grain), and several soil nutrients (nitrate, nitrite, phosphate, potassium, total nitrogen, and total phosphorus content) were measured with an automatic wet chemistry analyzer (Skalar 4000 SAN System, Segmented Flow Analyzer; Skalar, Breda, The Netherlands). Nitrate, nitrite, and phosphate contents were determined through colorimetry and potassium contents through flame photometry in the wet analyzer. To

TABLE 1. Characterization of high-quality and poor-quality habitat sites.

Site type	Nitrate (ppm)	Nitrite (ppb)	Phosphate (ppb)	Potassium (ppb)	Total P (ppm)	Total N (ppm)	Total cover (%)	Bare soil (%)	Lichens and moss cover (%)	Litter cover (%)	Annual cover (%)	Perennial cover (%)
High	1.20 ± 0.10	22.63 ± 1.73*	49.22 ± 4.80*	6.55 ± 0.56*	2.00 ± 0.16*	19.65 ± 1.65*	67.07 ± 0.80*	10.25 ± 0.72*	20.57 ± 0.76	10.11 ± 0.46	16.76 ± 0.69*	29.90 ± 0.76
Poor	1.24 ± 0.24	17.50 ± 1.53	35.40 ± 1.73	4.92 ± 0.26	1.33 ± 0.09	12.70 ± 1.18	47.43 ± 0.80	22.63 ± 0.74	16.96 ± 0.64	11.63 ± 0.40	6.36 ± 0.46	26.50 ± 0.72

Notes: For soil fertility values, $N = 60$. For patch attributes, $N = 480$. Values are expressed as mean \pm SE. An asterisk indicates significant differences (at $P < 0.05$) between high-quality and poor-quality habitat patches. See Appendix C for detailed statistical analyses of these data.

determine total phosphorous and total nitrogen contents, soil samples were first digested according to the Kjeldhal method and determined through colorimetry afterwards (Radojevic and Bashkin 1999). Since changes in habitat quality are in general intimately linked to habitat fragmentation, we included the factor fragmentation in the comparison between high- and poor-quality sites to check for possible differential effects of fragmentation in soil properties and fertility (i.e., higher fertility in high-quality habitat and large patches). As expected, in all the cases, significant differences in soil and patch properties were exclusively due to habitat quality, and no significant effect of fragmentation was found (see Appendix B for complete statistics of habitat quality data).

In March 2005, we established two plots per fragment, one for high-quality habitat and one for poor-quality habitat, and their coordinates were recorded with a GPS. Plots were located at least 2 m apart from the edge of each fragment to prevent border effects. Each plot contained at least 40 reproductive individuals of *Centaurea hyssopifolia*, resulting in plots of 15×15 m average (225 m^2 ; range = $220\text{--}420 \text{ m}^2$). Forty plants were randomly selected within each plot and tagged, rendering a total of 480 plants. A laser distance meter (Leica Disto lite 5, Leica Geosystems, Heerbrugg, Switzerland) was used to position all plants in each plot. Mean plant size (estimated as a function of plant diameter and height: $[h\pi(d/2)^2]$, where h is the maximum height and d is the maximum diameter) was $18880 \pm 800 \text{ cm}^3$ (range = $146\text{--}140928 \text{ cm}^3$). Initial plant size did not differ between large and small fragments ($F_{1,476} = 0.015$, $P = 0.981$) nor between high- and poor-quality habitat plots ($F_{1,476} = 0.01$, $P = 0.983$).

Water manipulation was conducted by means of an irrigation experiment performed in May and June of both study years to generate two water availability levels. These months were selected for two reasons. First, the projections of the most likely future climate scenario for the Mediterranean area indicate significant reductions in mean annual rainfall, primarily decreasing precipitation during spring and summer (Christensen et al. 2007). Actually, 2005 and 2006, which were drier than average years, exhibited significantly drier spring and summer months (Appendix A). Second, these months coincide with the growth peak and reproduction events in this species, so plants are expected to be highly

sensitive to changes in climatic conditions during this period. Each plot was divided into two contiguous halves containing 20 plants, which were assigned to the dry (non-watered) or mesic (watered) treatments (20 plants \times 12 plots = 240 plants in each water treatment). Each watered plant was at least 2 m from the closest non-watered plant. Irrigation consisted of adding 1 L of water per plant and application time (see Appendix C for a detailed description of the irrigation experiment, including total amount of water added). Water was carefully added to avoid runoff and to maximize absorption by the soil. A $50 \times 50 \text{ cm}$ (0.25-m^2) rigid frame was placed around each plant so that the entire surface was watered; each application was equivalent to 4-mm rainfall events. This protocol simulated a typical spring/summer rainfall in this area, according to local climatic data. Irrigation was carried out at 5- to 6-d intervals. The non-watered (dry treatment) plants received ambient rainfall (equivalent to future drier scenarios due to the opportunity of very dry spring conditions of the study years), and the watered plants received ambient rainfall plus the added water (equivalent to a typical year). A pluviometer was established in the field site, and rainfall was daily monitored from the beginning of the experiment. Water was added to reach the median of the long-term series in each month (Appendix C). In total, 480 plants were considered in 2005 and 240 in 2006 after random subsampling.

Data collection

Soil water content (SWC, as a percentage) was monitored in 7–20 plants per treatment (three measurements per plant under shrub canopy) with a soil moisture sensor (ThetaProbe, Delta-T Devices, Cambridge, UK). Measurements were taken at the beginning of the irrigation experiment and at different times after an irrigation event in both study years.

Plant survival was monitored in all plants throughout the experiment. We labeled as dead those individuals presenting no photosynthetically active leaves (i.e., green and flexible leaves) and exhibiting loss of flexibility in the branches. These estimates were shown to be valid since plants presumed to be dead did not resprout or produce any new tissues in the succeeding year. Maximum height and maximum diameter were measured twice per year in all the plants. We estimated

relative growth in each study year as $(\ln(G_2) - \ln(G_1))/(t_2 - t_1)$, with G_1 and G_2 being plant size at time 1 and 2 (t_1 and t_2 , respectively).

Plant phenology was monitored in the two study years every 10–12 days throughout the flowering and fruiting period, from capitula formation to seed dispersion (late May–July), in all the plants. A total of five censuses were carried out in 2005 and four in 2006. In each census, the fraction of senescent and green leaves and the different reproductive phenophases were identified and estimated in each plant (all plants, i.e., 480 in 2005 and 240 in 2006, were examined in each census). The reproductive phenophases identified were closed capitula (capitula with no florets present), flowering capitula (with fresh florets), mature capitula (with senescent florets), and open capitula (after seed dispersion); data were expressed as percentages of total capitula of each plant. With these data, the following parameters were calculated for each plant and were used as the dependent variables: (1) flowering onset, as the days elapsed from 1 May to the detection of the first flowering capitula in each plant; (2) flowering duration, as the number of days each plant showed flowering capitula; (3) flowering peak, as the days elapsed from 1 May to the day on which the maximum percentage of flowering capitula was reached in each plant; (4) dispersion onset, as the days elapsed from 1 May to the detection of mature capitula in each plant, (5) dispersion duration, as the number of days each plant showed mature capitula, and (6) dispersion peak, as the days elapsed from 1 May to the day on which the maximum percentage of mature capitula was reached in each plant.

Total number of capitula of all plants was counted at the end of the reproductive period in each study year. Seven mature capitula per plant were randomly collected in 10 plants per treatment in 2005 (240 plants) and five plants per treatment in 2006 (120 plants). Plants were randomly selected within treatments. Capitula were dissected, and the numbers of viable, aborted, and parasitized seeds in each capitulum were counted. The sum of the three categories was calculated as an estimate of the flower number per capitulum. Since the fruit is monospermous, seed set was calculated as the proportion of flowers setting viable seeds. Average values of flower number, viable seeds, aborted seeds, and parasitized seeds was multiplied by the total number of capitula of each plant to obtain the total number of flowers, total viable seeds, total aborted seeds, and total parasitized seeds per plant. Finally, seven viable seeds per plant were randomly selected and individually weighed in a gram precision balance (0.1-mg precision).

Statistical analyses

The effects of treatments (fragmentation, habitat quality, and water availability) on the dependent variables (growth, phenology, and reproductive success data) were analyzed using a three-way nested ANOVA model. The model included fragmentation (F), habitat

quality (Q), and water availability (W) as main fixed factors. Each fragment was considered as a random factor nested within fragmentation level (fragment(F)), whereas plant size (volume) was taken as a continuous predictor. We also included all possible interactions between fixed factors. In this model, the effect of fragmentation (1 df) was tested against the random effect of fragment nested within fragmentation (4 df). The main effects of habitat quality (1 df) and water availability (1 df), as well as the interactions between fragmentation and habitat quality/water availability (i.e., $F \times Q$ or $F \times W$) were tested against the random effect of the interaction between fragment and habitat quality/water availability (fragment(F) \times Q or fragment(F) \times W). Finally, the interaction between habitat quality and water availability (Q \times W) and the three-factor interaction (F \times Q \times W) were tested against the term fragment(F) \times Q \times W, and the effect of plant size was nested within fragment and tested against the residual error (df = 450 in 2005 and df = 210 in 2006). Data for reproductive traits at capitulum level (flower number, viable seeds, aborted seeds, seed set, and seed mass) were pooled and averaged from the seven capitula/seeds collected for each plant. A separate analysis was performed for each study year. To test the effects of treatments on plant survival, the percentage of surviving plants from March 2005 to April 2006 was determined for each plot and treatment combination and was used as the response variable; the mean plant size per plot and treatment was also calculated and used as a covariate in the survival analysis. The dependent variables were checked for normality prior to analyses. All analyses were performed with Statistica 6.0 (Statsoft, Tulsa, Oklahoma, USA).

RESULTS

Soil water content

Soil water content was very low over the study period. At the onset of the irrigation experiment, no differences were found in SWC beneath plants from different treatments when comparing values from high- or poor-quality sites ($1.24\% \pm 0.12\%$ and $4.52\% \pm 0.32\%$, respectively; $F_{1,156} = 7.93$, $P = 0.067$) or watered and non-watered plants ($3.46\% \pm 0.41\%$ and $2.51\% \pm 0.30\%$, respectively; $F_{1,156} = 3.24$, $P = 0.150$; $N = 160$). Four days after an irrigation event in May, significant differences in SWC were found among watered and non-watered plants ($1.73\% \pm 0.14\%$ and $0.42\% \pm 0.07\%$, respectively; $F_{1,276} = 80.07$, $P < 0.001$) and among plants from high- and poor-quality sites with higher SWC for the former ($1.70\% \pm 0.16\%$ and $0.47\% \pm 0.05\%$, respectively; $F_{1,276} = 75.65$, $P < 0.001$; $N = 280$). The same was true four days after an irrigation event in June.

Survival and growth responses

Survival was high in all treatments (see Appendix D for mean values of all traits across treatments). However, we found a significant effect of fragmentation

TABLE 2. ANOVA results (*P* values) for the phenological traits in each study year.

Year	Fragmentation (F)	Habitat quality (Q)	Water availability (W)	F × Q	F × W	Q × W	F × Q × W	Plant size
Survival	0.046	0.013	NS	0.047	NS	NS	NS	NS
Growth								
2005	NS	0.049	NS	0.044	NS	NS	NS	NS
2006	NS	NS	NS	NS	NS	NS	NS	NS
Phenology								
Flowering onset								
2005	NS	0.014	NS	NS	NS	NS	NS	<0.0001
2006	NS	0.033	0.038	NS	NS	0.043	NS	0.04
Flowering duration								
2005	NS	NS	NS	NS	NS	NS	NS	<0.0001
2006	NS	NS	NS	NS	NS	NS	NS	0.002
Flowering peak								
2005	NS	0.031	0.029	NS	NS	NS	NS	<0.001
2006	NS	0.024	NS	NS	NS	NS	NS	0.047
Dispersion onset								
2005	NS	<0.0001	0.048	0.01	NS	NS	NS	<0.001
2006	NS	0.015	0.024	NS	NS	NS	NS	NS
Dispersion duration								
2005	NS	0.035	0.026	NS	0.042	NS	NS	<0.001
2006	NS	0.017	0.011	NS	NS	NS	NS	0.042
Dispersion peak								
2005	NS	0.005	0.03	NS	NS	NS	NS	0.014
2006	NS	0.037	0.038	NS	NS	NS	NS	NS
Senescent leaves (%)								
2005	NS	0.012	0.021	NS	NS	0.042	NS	<0.001
2006	NS	0.019	<0.001	NS	NS	NS	NS	0.047
Senescence duration								
2005	NS	0.01	0.027	0.047	NS	NS	NS	<0.001
2006	NS	0.005	0.008	NS	NS	NS	NS	0.003

Notes: In 2005, *N* = 480 plants, and in 2006, *N* = 240 plants. See *Results: Phenology* for details on the model and direction of effects. "NS" indicates not significant.

and habitat quality on plant survival (Table 2). Plant survival was significantly higher on large fragments and on high-quality habitat sites. Also, there was a significant interaction between these two factors, revealing significantly lower survival in plants from poor-quality habitat sites of small fragments. We found no effect of plant size on survival.

In 2005 there was a significant and negative effect of poor habitat quality on plant growth and a significant interaction between fragmentation and habitat quality: plant growth was significantly lower in plants from poor-quality habitat sites of small fragments (Table 2; Fig. 2). Surprisingly, no significant effects of treatments were found for plant growth in 2006. Plant size was not significantly related to plant growth in any study year.

Phenology

Percentage of flowering plants was high in both study years (96.3% in 2005 and 97.0% in 2006), and flowering extended from late May to late June. We found a significant effect of habitat quality on flowering onset in both years, revealing an advance in flowering onset in

the plants from poor-quality habitat sites (Table 2, Fig. 3). A significant effect of water availability and an interaction between habitat quality and water availability were also found in 2006, showing the greatest advance in flowering in non-watered plants from poor-quality habitat sites (Fig. 3). A significant effect of habitat quality on the peak of flowering was also found in both study years with plants from poor-quality habitat sites having the peak of flowering earlier than plants from high-quality habitat sites. A significant effect of water availability was found as well in 2006, showing an advance in the peak of flowering in non-watered plants (Table 2, Fig. 3). Finally, we did not find differences in flowering duration among treatments in any study year (Table 2, Fig. 3).

We found a significant effect of habitat quality and water availability on dispersion onset in both study years (Table 2, Fig. 3). Dispersion started earlier in plants from poor-quality habitat sites and in non-watered plants. A significant interaction between habitat quality and fragmentation was also found in 2005, with plants from high-quality sites of large fragments starting

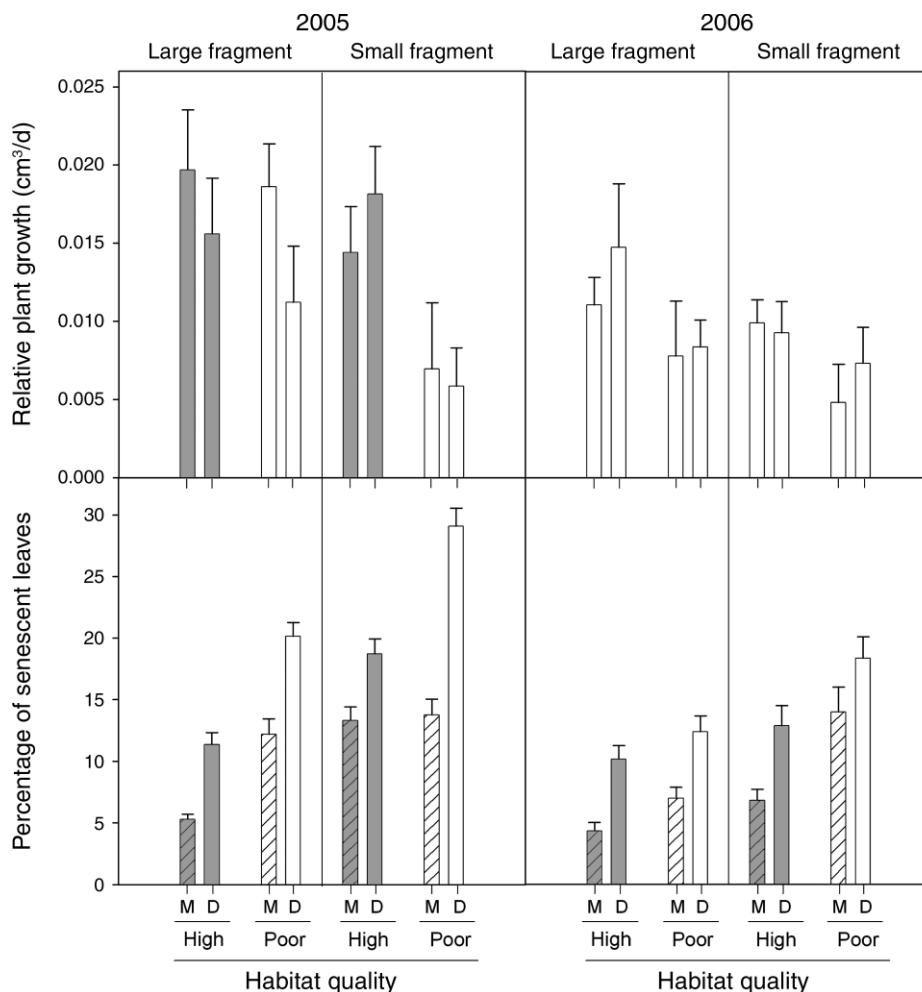


FIG. 2. Relative plant growth and percentage of senescent leaves of *Centaurea hyssopifolia* plants compared across treatments for 2005 and 2006. Values are mean + SE in each treatment. Each half of a panel corresponds to data from large and small fragments. Abbreviations are: M, mesic treatment (watered plants); D, dry treatment (non-watered treatments). Different shading and patterns among bars show significant main effects.

dispersion later. Dispersion lasted longer in plants from poor-quality habitat sites and in non-watered plants (Table 2, Fig. 3). Additionally, an interaction between fragmentation and water availability was found in 2005. This interaction revealed that dispersion was longer in non-watered plants from small fragments. Similarly, we found an effect of habitat quality and water availability in the moment of dispersion peak: plants in poor-quality habitat sites and non-watered plants reached the peak earlier in both study years (Table 2, Fig. 3).

The percentage of senescent leaves and duration of senescence was significantly higher in non-watered plants and in plants from poor-quality habitat sites (Table 2, Fig. 2). Also, in 2005 the amount of senescent leaves was larger in non-watered (dry treatment) plants from poor-quality habitat sites, as shown by the interaction between these two experimental factors, and senescence duration was longer in poor-quality habitat sites of small fragments. Finally, we found a

highly significant effect of plant size on most of the phenological traits (Table 2).

Reproductive traits

We found a significant effect of fragmentation on the number of flowers per capitulum in 2006: plants from small fragments had lower numbers of flowers (Table 3, Fig. 4). An interaction between habitat quality and water availability also was found, with a higher number of flowers per capitulum in watered plants from poor-quality habitat sites and in non-watered plants from high-quality habitat sites. The number of viable seeds per capitulum was significantly higher in plants from large fragments in both study years (Table 3, Fig. 4). In 2006, there was also a significant effect of water availability, so the number of viable seeds was significantly higher on watered plants. In addition, the triple interaction among factors revealed that the effect of fragmentation was visible only in the watered plants, i.e.,

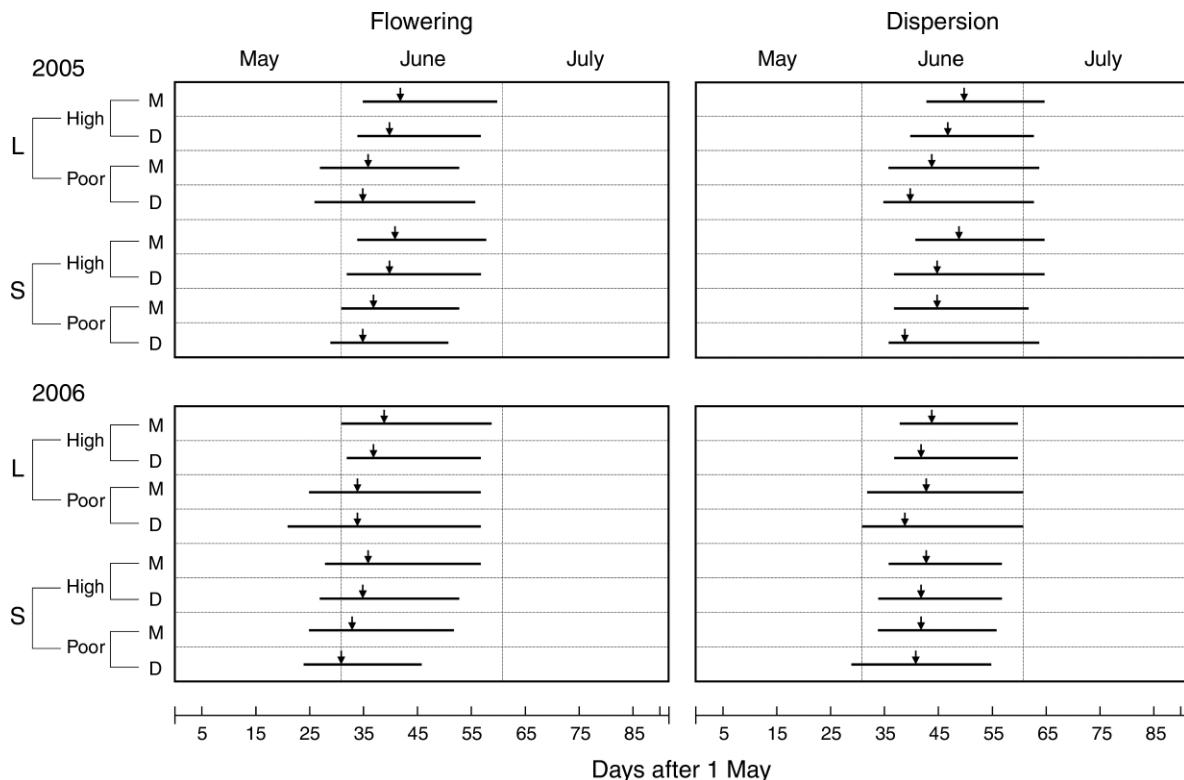


FIG. 3. Phenology of *Centaurea hyssopifolia* in the different treatments. Black bars represent mean flowering or dispersion onset and duration. The arrows indicate the peak of each phenophase. Abbreviations are: M, mesic treatment (watered plants); D, dry treatment (non-watered treatments); High, high-quality habitat sites; Poor, poor-quality habitat sites; L, large fragment; S, small fragment.

the number of viable seeds was higher on plants from large fragments, but only in the watered plants. The number of aborted seeds per capitulum was significantly higher in non-watered plants only in 2006 (Table 3). Also, the interaction between fragmentation and water availability revealed a higher number of aborted seeds in non-watered plants from large fragments. No effects of treatments were found in the number of parasitized seeds per capitulum. Seed set was significantly higher in large fragments in 2005 (Table 3, Fig. 4). No significant effects of plant size were found for reproductive traits at the capitulum level.

We did not find significant effects of treatments in any of the study years for the reproductive traits at the plant level (total number of capitula, total number of flowers, total viable seeds, total aborted seeds, and total parasitized seeds; Table 3). Finally, there was a negative effect of fragmentation on seed mass in 2005: seed mass was significantly higher on plants from large fragments (Table 3, Fig. 4). There also was a significant interaction between fragmentation and water availability, so that seed mass was higher in watered plants from large fragments, and an interaction between habitat quality and water availability, in which seed mass was higher in watered plants from high-quality habitat sites. Plant size

had a highly significant effect on reproductive traits at the plant level.

DISCUSSION

Fragmentation, habitat quality, and water availability profoundly and interactively affected the performance of *Centaurea hyssopifolia*. Interestingly, the impact of these global change drivers differed among traits. While growth and phenology were mainly affected by habitat quality and reductions in rainfall, reproduction was mainly affected by fragmentation.

Female reproductive output was negatively affected by fragmentation in both years. Our results showed a 25–66% decrease in the number of viable seeds at the capitulum level in plants from small fragments, which agreed with a large body of studies showing negative effects of fragmentation on the reproductive output of plant species (e.g., Oostermeijer et al. 1994, Agren 1996, Lienert and Fischer 2003, Leimu et al. 2006). This is especially striking because most of our small fragments have been created only after 1984 (see Fig. 1), as a pernicious side effect of the common agriculture policy of the European Union (Concepcion et al. 2008). It is known that plants in small fragments may face both ecological and genetic problems. On the one hand, small populations may experience increased inbreeding and

TABLE 3. ANOVA results (*P* values) for the reproductive traits at the capitulum level and the plant level.

Reproductive trait	Fragmentation (F)	Habitat quality (Q)	Water availability (W)	F × Q	F × W	Q × W	F × Q × W	Plant size
Flower number per capitulum								
2005	NS	NS	NS	NS	NS	NS	NS	NS
2006	0.037	NS	NS	NS	NS	0.037	NS	NS
Viable seeds per capitulum								
2005	0.01	NS	NS	NS	NS	NS	NS	NS
2006	0.04	NS	0.022	NS	NS	NS	0.046	NS
Aborted seeds per capitulum								
2005	NS	NS	NS	NS	NS	NS	NS	NS
2006	NS	NS	0.008	NS	0.005	NS	NS	NS
Parasitized seeds per capitulum								
2005	NS	NS	NS	NS	NS	NS	NS	NS
2006	NS	NS	NS	NS	NS	NS	NS	NS
Total capitula								
2005	NS	NS	NS	NS	NS	NS	NS	<0.001
2006	NS	NS	NS	NS	NS	NS	NS	<0.001
Total flower number								
2005	NS	NS	NS	NS	NS	NS	NS	<0.001
2006	NS	NS	NS	NS	NS	NS	NS	<0.001
Total viable seeds								
2005	NS	NS	NS	NS	NS	NS	NS	<0.001
2006	NS	NS	NS	NS	NS	NS	NS	NS
Total aborted seeds								
2005	NS	NS	NS	NS	NS	NS	NS	<0.001
2006	NS	NS	NS	NS	NS	NS	NS	<0.001
Total parasitized seeds								
2005	NS	NS	NS	NS	NS	NS	NS	NS
2006	NS	NS	NS	NS	NS	NS	NS	<0.001
Seed set								
2005	0.007	NS	NS	NS	NS	NS	NS	NS
2006	NS	NS	NS	NS	NS	NS	NS	NS
Seed mass								
2005	0.012	NS	NS	NS	0.005	0.002	NS	NS
2006	NS	NS	NS	NS	NS	NS	NS	NS

Notes: In 2005, $N = 240$ plants, and in 2006, $N = 120$ plants. See *Results: Reproductive traits* for details on the model and direction of effects. "NS" indicates not significant.

decreased genetic variation due to genetic drift and founder effects (Ellstrand and Elam 1993, Fischer et al. 2003). On the other hand, disruptions in plant–pollinator interactions may reduce plant fitness due to pollination limitation, as pollinators may be less attracted to small plant patches (Sih and Baltus 1987, Aguilar et al. 2006).

In our study, pollinator availability may be one of the mechanisms explaining the reduced reproductive output observed in the plants inhabiting small fragments. Despite the fact that *Centaurea hyssopifolia* is a generalist-pollinated species (Luzuriaga et al. 2006), it has been shown that generalist- and specialist-pollinated plant species show similar reproductive susceptibility to habitat fragmentation (Ashworth et al. 2004, Aguilar et al. 2006). Generalist pollinators in small fragments may visit different plant species in the same flight and also may stay longer in different flowers within the same plant (Schulke and Waser 2001, Duncan et al. 2004), therefore reducing the availability of outcross

conspecific pollen. This fact is especially relevant in self-incompatible, obligate outbreeder species such as *C. hyssopifolia* (Ellstrand and Elam 1993, Aguilar et al. 2006, Leimu et al. 2006), which have been shown to be more sensitive to plant–pollinator disruptions than self-compatible species (Aguilar et al. 2006, Leimu et al. 2006). Genetic factors also may have contributed to the observed reduced reproduction in small fragments. In populations of low genetic diversity, outcrossing between related plants can result in high levels of biparental inbreeding (Husband and Schemske 1996, Fischer et al. 2003), which eventually may reduce the availability of suitable mating partners and thus fitness (Ellstrand and Elam 1993, Leimu et al. 2006). Although our study species is a perennial and fragmentation is a relatively recent process in the study site, we found traits that are frequently used as indicators of inbreeding depression, such as lower survival of plants or lower seed mass (Lienert 2004).

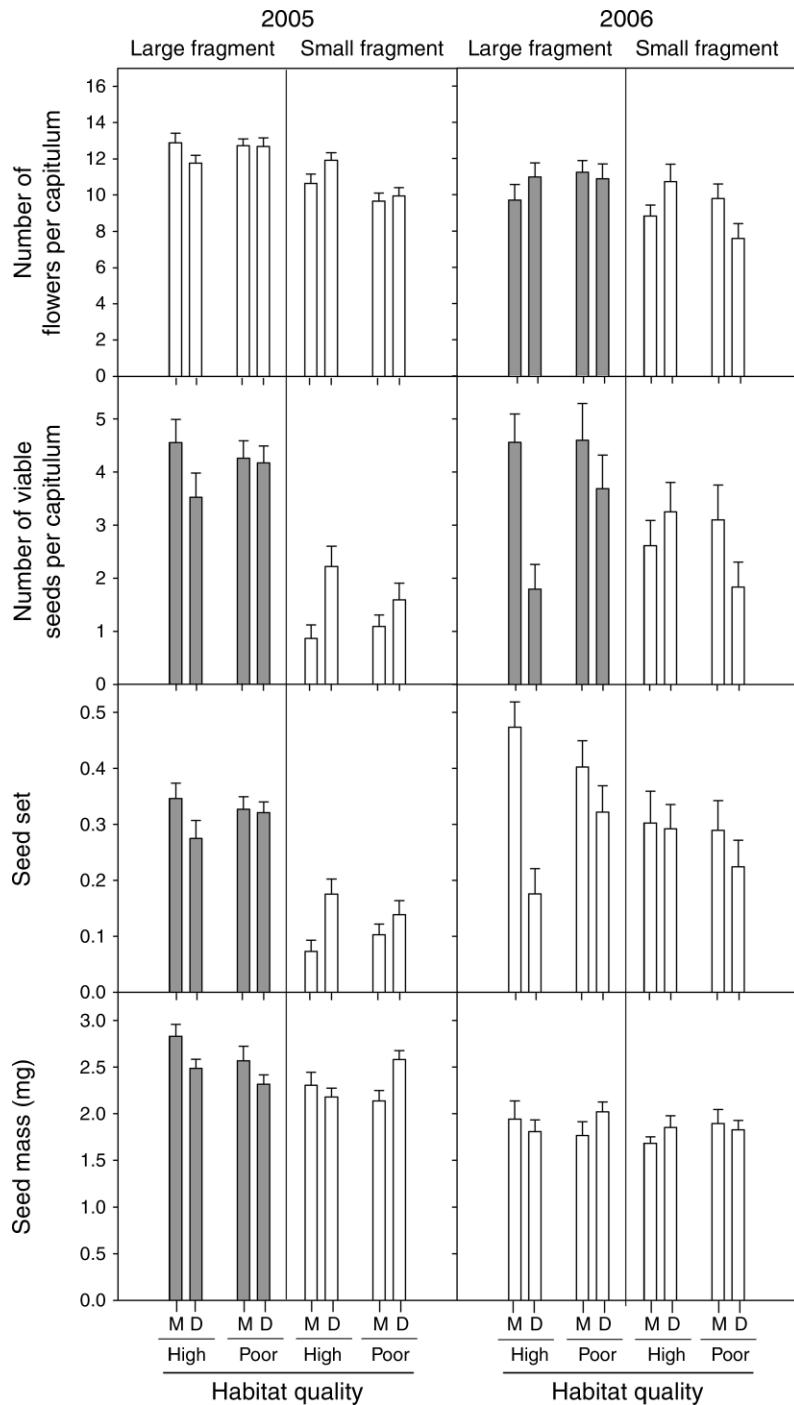


FIG. 4. Number of flowers per capitulum, number of viable seeds per capitulum, seed set, and seed mass in 2005 and 2006. Values are means \pm SE in each treatment. Each half of a panel corresponds to data from large and small fragments, respectively. Abbreviations are: M, mesic treatment (watered plants); D, dry treatment (non-watered treatments). Different shading among bars shows significant main effects.

It must be noted that the effects of fragmentation were observed mainly at the capitulum level. At the plant level, there was a great effect of plant size on the absolute reproductive output, which is a common pattern in many plants (Herrera 1991, Ollerton and Lack 1998, Luzuriaga

et al. 2006). The numbers of flowers, capitula, and seeds produced by a plant greatly depend on the availability of resources, which is mainly determined by plant size. Furthermore, the lack of effect of fragmentation at the plant level may be due to the relatively higher (but not

significant) number of capitula per plant in the small fragments compared to the large ones (see Appendix D for total capitula numbers). At the capitulum level, seed production may be more determined by factors other than plant size, such as interactions with pollinators and pollen quality (Agren et al. 2008). Collectively, our results may be interpreted as a change in the efficiency of seed production triggered by genetic and ecological factors. Although the implications of these findings in the long run are unclear, they are likely to represent early symptoms of potentially detrimental effects of fragmentation in the fitness of our study species.

In addition to these changes in the reproductive output of *C. hyssopifolia*, we also observed changes in the onset, duration, and peak of reproductive and dispersal events in response to habitat quality and climate change in both study years. Based on its well-known variation with annual weather, it is expected that plant phenology will be one of the most responsive and easily observable traits that change in response to climate (Badeck et al. 2004, Parmesan 2006). In addition and related to the known major role of water in semiarid ecosystems, altered phenological patterns associated with reductions in rainfall were also expected. In this sense, it is worth mentioning that both study years had drier-than-average springs, matching the predictions for this region. Accordingly, other stressful abiotic conditions such as those experienced in the poor-quality habitat sites (e.g., lower nutrient content) may also elicit changes in phenology (Sandvik and Totland 2000, Franks et al. 2007). Under resource limitation, plants may either invest in reproduction, which may limit vegetative growth, or cope with stress through tolerance (e.g., increasing water or nutrient use efficiency; Cohen 1976, Fox 1990, Franks et al. 2007). When environmental conditions (e.g., water availability or habitat quality) are benign, we may expect a delay in flowering and other phenophases because plants that spend more time growing will have more resources and thus will produce more flowers and seeds (Cohen 1976, Franks et al. 2007). However, when conditions are resource limiting (e.g., drought or reduced quality habitat sites), plants may advance their phenophases in order to mature seeds before conditions deteriorate too much, thereby ensuring reproduction but limiting growth. Our results agree with a stress escape strategy, as stressed plants (i.e., non-watered plants and plants in poor-quality habitat sites) tended to advance most phenophases. This fact also coincides with the lower relative growth detected in plants from the poor-quality habitat sites in 2005. Interestingly, abiotic stress did not result in lower reproductive success (see Table 3). Only in 2006 did we observe a lower number of viable seeds in non-watered plants, which matches with recent evidence relating altered phenological patterns with a decoupling of flowering and pollination activity (Badeck et al. 2004, Parmesan 2006). We are unable to determine whether this change represents a passive plastic response of *C.*

hyssopifolia to stress conditions or a genetically based evolutionary shift promoted by selection for early-flowering genotypes. The potential for rapid evolution is especially important for plant species inhabiting fragmented landscapes, as habitat fragmentation may prevent plants from migrating toward sites of more favorable climate envelopes and the lower genetic variation in fragmented populations may decrease the evolutionary potential of species to adapt to changing environments (Leimu et al. 2006, Parmesan 2006, Franks et al. 2007).

Our study also detected significant interactions among global change drivers affecting several traits. Synergy among multiple drivers of change occurs when the net impact of two drivers is significantly higher than the sum of the two operating independently (Sala et al. 2000). For example, interactions between fragmentation and habitat quality led to lower survival and lower relative growth in plants from poor-quality habitat sites of small fragments. Eventually, this may translate into lower average plant size and further reductions in reproductive output, as plant size was a good predictor of plant phenology and total reproductive output in both study years. Despite the relatively small number of interactions among global change drivers found here, the fact that phenology was mainly affected by water availability and habitat quality and reproductive traits were mainly affected by fragmentation highlights the importance of including all relevant drivers affecting a given study system to reach sound conclusions on the impact of global change.

In general, our results showed consistent patterns between years. For example, flowering phenology and senescence traits responded in a very similar way to changes in habitat quality and water availability in both study years. However, we found interannual differences in the effects of fragmentation on the reproductive traits. Most studies on fragmentation effects carried out over time periods longer than one season show that the effects of population size on plant reproductive output changed widely between years differing in climatic conditions (e.g., Molano-Flores et al. 1999, Morgan 1999, Murren 2002). Environmental conditions may affect both the abundance and composition of the pollinator and herbivore community and therefore influence reproductive output (Aizen and Feinsinger 2003). In our study, the number of viable seeds was negatively affected by fragmentation in both years, but differences between years were found for flower number and seed set. This suggests the existence of other ecological factors operating beyond genetic factors, as inbreeding effects would presumably be consistent across years.

In conclusion, our results show that impacts of global change cannot be predicted from single-factor studies not only because of interactions between drivers but also because different drivers affect different aspects of plant performance. Cumulative effects and stronger interactions among drivers can be expected in the long run, further emphasizing the need for considering several

drivers and traits simultaneously in studies exploring global change impacts. The negative effect of fragmentation on reproductive traits, despite the relative recentness of this process in our study site, together with the spatial limitation of gypsophytes to gypsum soils points to a high vulnerability of these plants to global change.

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APPENDIX A

Climatic conditions at the study site (*Ecological Archives* E090-181-A1).

APPENDIX B

Summary results of the two-way ANOVA to test for the effects of fragmentation and habitat quality on soil fertility and patch attributes (*Ecological Archives* E090-181-A2).

APPENDIX C

Description of the irrigation experiment (*Ecological Archives* E090-181-A3).

APPENDIX D

Study traits for 2005 and 2006 in the different treatments (*Ecological Archives* E090-181-A4).