



Hydroperiod length as key parameter controlling seed strategies in Mediterranean salt marshes: The case of *Halopeplis amplexicaulis*

Elena Estrelles^a, Josefa Prieto-Mossi^a, M. Carmen Escibá^{b,c}, Inmaculada Ferrando^{b,c}, Pablo Ferrer-Gallego^{b,c}, Emilio Laguna^b, Ana M. Ibars^a, Pilar Soriano^{a,*}

^a Botanical Garden—ICBiBE, Universitat de València, Quart 80, 46008, Valencia, Spain

^b CIEF—Centre for Forestry Research and Experimentation, Generalitat Valenciana, Comarques del País Valencià 114, Quart de Poblet, 46930, Valencia, Spain

^c VAERSA, Avda. Cortes Valencianas 20, 46015, Valencia, Spain



ARTICLE INFO

Edited by Fei-Hai Yu

Keywords:

Maternal environmental effect
Annual plants
Seed dormancy
Bet-hedging
Opportunistic germination strategy
Halopriming

ABSTRACT

The comprehension of plant biology and the response to the environment is fundamental to achieve the optimal skills to manage and conserve the fine equilibrium between biotic and abiotic parameters regulating natural biodiversity in salt marshes. The behaviour of annuals living in these stressful conditions is poorly understood and constitutes a good model for a better understanding of this relationship. We thus identified the determinant environmental factors involved in population survival of *Halopeplis amplexicaulis*, a threatened annual species inhabiting salt marshes. To achieve this objective, maternal climatic parameters were analyzed in seeds collected in different years, and correlated to seed dimensions (length, width and length/width ratio) and mass, and also to germination responses of the next generation. Our results proved a direct correlation between seed factors and the maternal environment determining the hydroperiod length, particularly autumn and winter temperatures, responsible of life cycle extent. Population survival is ensured by a narrow adaptation to climatic conditions through a natural halopriming of seeds. The combination of the two complementary germination strategies verified (opportunistic vs. bet-hedging) depending on the climatic conditions of the ripening year, is revealed as a density regulation mechanism controlling seed bank dynamics.

1. Introduction

Salt marshes show a fine equilibrium between biotic and abiotic parameters regulating natural biodiversity (Mudd and Fagherazzi, 2016). These habitats are subjected to strong fluctuations on species composition depending to environment (Noto and Shurin, 2017). Hydroperiod regime, depending to the specific precipitation and evaporation patterns, is one of the main environmental factors regulating the creation of niches in wetland plants (Foti et al., 2012). The potential effects of climate change on vegetation changes have renewed the attention to the effects of environmental parameters on alteration in biodiversity and plant reproductive output (Del Cacho et al., 2013), particularly in wetlands.

Knowledge of reproductive biology, particularly phenology, seed features and germination response to environmental conditions, is a crucial point to understand post-germinative behaviour (Baskin and Baskin, 1998; Donohue et al., 2010) affecting the survival of future generations, and therefore the maintenance of the natural population. Long et al. (2015) compiled data about the abiotic and biotic factors

that influence seed behaviour within a range of environments, and pointed out the necessity of future research to identify which traits drive seed persistence. Indeed, maternal effects, seed dormancy patterns, longevity of seeds in the soil seed bank, and survival of seedlings after germination become relevant issues to understand and model population dynamics, especially for endangered plants (Fenner, 2000; Iriondo et al., 2009). This fact has been particularly remarked in annuals and other short-lived species of dry habitats, often chosen as an example of bet-hedging strategies (Philippi, 1993a,b).

For this study, we have chosen *Halopeplis amplexicaulis* (Vahl) Ung.-Sternb., the only European and Mediterranean representative of this genus, as a model, which inhabits salty habitats (endorheic ponds), throughout the Mediterranean region, with a Mediterranean-Iranoturanian connection from Portugal to Turkey through the Near East (Aellen, 1967; Jalas and Suominen, 1980; Blanché, 1990). This annual pioneer plant grows in territories with ombrotypes that range from dry (in some Spanish populations) to ultra-hyperarid (in some regions of Jordan), colonizing soils rich in salts and showing low competitiveness with other species, mainly perennials (Tremblin, 2000;

* Corresponding author.

E-mail address: pilar.soriano@uv.es (P. Soriano).

<https://doi.org/10.1016/j.flora.2018.10.006>

Received 30 May 2018; Received in revised form 18 October 2018; Accepted 21 October 2018

Available online 24 October 2018

0367-2530/© 2018 Elsevier GmbH. All rights reserved.

Tremblin and Binet, 1984). Due to the extreme rareness and severe fluctuations in population size, this species has been listed as Vulnerable on the Spanish Red List of Vascular Plants (Suárez et al., 2007), and strictly protected at regional level (Aguilella et al., 2010; Laguna et al., 2013). At the European level, Habitats Directive 92/43/EEC (European Commission, 2013) considers the habitats of this species, 'Mediterranean salt steppes' (*1510) and 'Salicornia and other annuals colonising mud and sand' (1310), to be of interest for conservation.

The ecophysiological traits and seed germination of some *Halopeplis* species have been focus of study (Tremblin and Binet, 1982; Mahmoud et al., 1983; Khan and Weber, 2008). Diverse authors provided detailed observations of *H. amplexicaulis* life cycle and phenology, stating substantial divergences (Suárez et al., 2007; Tremblin, 2000). Additionally, several studies indicated conflicting behaviour regarding seed dormancy level of this species (Albert et al., 2002; Ferrer-Gallego et al., 2013; Santo, 2013). Hence, neither the germination strategy of this species nor regulatory factors are clearly defined.

All the above considerations led us to undertake a study of the morphological variability and the differences in germination responses in seeds of *H. amplexicaulis* among different accessions with an initial hypothesis aimed to reveal inter-annual variability and check potential causes. Therefore, the main objective of this study was to provide a clear answer to the following questions:

1) Is the germinative behaviour a characteristic of the species or does it depend on the accession?

2) Are seed features and germination pattern affected by the environment?

2a) Are the specific climatology of the ripening year (maternal environment) potentially correlated to the different seed parameters and behaviour?

2b) Does seed germination be affected by other environmental factors than climate, such as salinity or light in natural environments?

3) Does seed age be a key parameter for seed germination of this species?

To address the above questions, we have analysed the specific climate pattern of the ripening years of each used accessions, and also all the seed features, both morphological characters and germination response under diverse conditions. Furthermore, the potential causes of the detected divergences have been discussed taking into account the specific climate conditions.

2. Material and methods

2.1. Plant material

Four seed accessions from different years were used, all of which were collected from the only known Valencian population (the Natural Park El Hondo, Alicante, Spain, UTM 30SXH9428). Mature and apparently viable seeds were timely harvested on the dates specified in Table 1. From our own field observations, we found that the fruiting period lasted until August and dispersion of mature seeds occurred in August and September. Accessions were maintained under laboratory conditions (20 °C; 40% relative humidity on average) until experiments started, except the sample collected in 2013, which was tested immediately after collecting. Storage time was detailed in Table 1.

Table 1
Seed collecting dates, storage time- (St) and seed features for the studied accessions.

Accession	Coll. date	St (years)	Length (mm)	Width (mm)	L/W	Seed mass (g)
A	17/09/2003	9	0.85 ± 0.05a	0.68 ± 0.04a	1.25 ± 0.07b	0.13 ± 0.02a
B	16/08/2010	3	0.79 ± 0.06b	0.61 ± 0.05b	1.31 ± 0.07a	0.11 ± 0.02b
C	04/08/2011	0.5	0.71 ± 0.08c	0.55 ± 0.05c	1.30 ± 0.09a	0.08 ± 0.02c
D	29/09/2013	0	0.80 ± 0.07b	0.61 ± 0.06b	1.31 ± 0.13a	0.10 ± 0.08b

Within the same variable, the same letters indicate no significant differences.

Different accessions were selected and then used in the diverse tests carried out according to seed availability.

2.2. Climatic characterisation of the study area

The studied locality where this species grows, with a mean annual temperature of 18.3 °C and precipitation of 291 mm (Catral, Alicante, Spain), corresponds to a Mediterranean xeric-oceanic bioclimate (Rivas-Martínez and Rivas-Saenz, 1996-2009). The aridity index (Ia) in this locality had a mean value of 0.31, and was thus categorized as a semiarid zone (UNEP, 1997). Annual temperatures and precipitation averages for a 31-year period (1982–2012) were provided by climate-data.org (<http://en.climate-data.org/location/177181/>). The meteorological station network of the IVIA (Valencian Institute of Agronomical Research) provided specific data for the period corresponding to maternal environment (monthly averages) from which seeds were collected of each studied accession. Data available on web page: <http://riegos.ivia.es/datos-meteorologicos>. The analysed periods were October 2002 – September 2003, October 2009 – September 2010, October 2010 – September 2011 and October 2012 – September 2013. With this specific data of temperature and precipitation, diverse climatic parameters, potentially associated to hydroperiod length, were calculated to characterize the environment and check the potential correlation with seed responses. All the parameters considered in this study are compiled in Appendix A.

Water balance graphs for each period were also plotted using the diagnosis tool provided by the Phytosociological Research Center (Rivas-Martínez and Rivas-Saenz, 1996-2009). This graph type represents lines of precipitation (supply of water) and potential evapotranspiration (natural demand for water) and provides information of water budget at the station. It is an easy manner to identify the water saturation or deficit periods. When the water balance graphs were analysed, we observed that the imbibition season there begins with autumn rains, usually around mid-November, while the water deficit starts at the end of February.

A hierarchical cluster analysis was carried out to classify the different climatic groups that corresponded to the studied periods. Monthly temperature and precipitation data and other climate parameters, calculated from them, were considered. Dissimilarities are defined by Squared Euclidean distance and the combination of clusters is based on Ward's Method.

2.3. Seed characterisation

To detect inter-annual variation and correlate seed morphology to climatic conditions, seed dimensions (length, width and length/width ratio) were measured on 100 seeds by the ImageJ, image analysis software (Rasband, 1997–2014) and seed mass was calculated using an Orion Cahn C-33 microbalance.

2.4. Seed germination tests

Seeds were sown in 5.5 cm Petri dishes, with a solution of 0.6% agar in four replications per assay of 20–25 seeds per plate, according to seed availability. Tests were carried out in Sanyo incubators with

temperatures controlled within ± 1 °C. Illumination was provided with daylight fluorescent tubes ($60\text{--}150\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$). The germination criterion was radicle emergence, and germinated seeds were discarded from dishes. The span of the test varied from 30 to 120 days until germination was stabilized and no further germination was observed. The seed response was expressed as final percentages of germination, and the T_{50} values (time required for 50% of the final germination percentage) were calculated according to Thanos and Doussi (1995).

The response to temperature was analysed for all the studied accessions (A, B, C and D) in an alternating temperature regime (25/15 °C) and its corresponding constant regime (20 °C) with a 12-h photoperiod. Additionally, the effect of different temperature (15, 20, 20/10, 25/15, 25/20 °C) and light regimes (darkness and a 12-hour daily photoperiod) were analysed with two of the four accessions (A and C). The germination done under dark conditions was recorded under safe light, provided by two green luminescent lamps (Philips TL-D 18W/17) supplemented with a green filter (Roscolux ref. no. 90), with a maximum transmission percentage at wavelengths from 500 to 540 nm.

In addition to the germination percentage and velocity, relative light germination (RLG) and relative alternating temperature germination (RATG) were also calculated to a better comprehension of seed behaviour under temperature and illumination variations. The RLG index which states the light requirement for seed germination was calculated by dividing the germination percentage recorded in the light (12 h light/12 h dark cycle) by the sum of the germination percentages observed in light and in darkness. The values vary from 0 (germination only in darkness condition) to 1 (germination only in light). Milberg et al. (2000) proposed this index, according to the formula:

$$\text{RLG} = \text{Gl}/(\text{Gd} + \text{Gl})$$

where Gl is the germination percentage in light, and Gd is the germination percentage in darkness.

The RATG index, which measures the requirement for alternating temperature, was calculated according to Aud and Ferraz (2012), by dividing the percentage of germination under constant temperature (20 °C) by the sum of the germination percentages at constant temperature (20 °C) and alternating temperatures (25/15 °C). The values vary from 0 (germination only at alternating temperature) to 1 (germination only at constant temperature).

A halopriming treatment was also performed in seeds that showed a slower germination (accessions B and C) to check the potential stimulating effect on germination. Two samples of seeds were pretreated in 5.5 cm Petri dishes with 500 mM NaCl on filter paper and incubated at two different temperatures, 25/15 and 20 °C, under dark conditions. After 7 days, the solution was drained and seeds were allowed to dry slowly under ambient conditions to simulate the natural process after short rain events. After 14 days of dry storage under room conditions, seeds were sown in agar, as described previously, and were incubated according to the same temperature regime, 25/15 and 20 °C respectively, with a 12/12 h photoperiod.

Table 2

Germination response, percentage (G%) and velocity (T_{50}) at 25/15 and 20 °C with a 12/12 h photoperiod and relative alternating temperature germination (RATG) values for the tested accessions.

Condition	Parameter	Accession			
		A	B	C	D
25/15 °C	G %	99.0 \pm 2.0	93.0 \pm 5.0	95.0 \pm 7.1	93.0 \pm 3.8
	T_{50}	4.6 \pm 0.1a	17.0 \pm 3.7b	17.6 \pm 4.6b	4.0 \pm 0.5a
20 °C	G %	4.0 \pm 3.3a	15.0 \pm 4.1ab	17.5 \pm 5.0b	31.8 \pm 8.0c
	T_{50}	2.8 \pm 2.9a	2.9 \pm 0.4a	3.0 \pm 0.5a	10.1 \pm 7.1b
RATG		0.04 \pm 0.03c	0.14 \pm 0.04b	0.15 \pm 0.04b	0.25 \pm 0.05a

Within the same variable, the same letters indicate no significant differences.

2.5. Data analysis

All the results were expressed as mean \pm SD. The data were statistically analysed using SPSS Version 15.0 for Windows. Descriptive statistics were graphically outlined through box plots.

Analysis of variance (ANOVA) was used to assess the significance of the observed effects ($P < 0.05$) (Khan and Rayner, 2003). Percentages were arcsine-transformed before the statistical analysis to ensure homogeneity of variance. One-way ANOVA and Tukey's HSD tests were run to compare the differences among treatments. A three-way ANOVA was applied to check the possible interactions, between seed accession, temperature regime and light conditions.

The analysed aspects, both morphological (length, width and mass) and germinative (germination, percentage and velocity, at 20 and 25/15 °C, and RATG), from seeds collected in different years were statistically correlated with the specific climatic parameters (Appendix A) measured during the plant growing and seed maturation period. To check this potential relationship a Spearman's correlation was applied to seed and climatic parameters.

3. Results

3.1. Seed characterisation

Seeds show an elliptic to obovate outline, with a smooth and translucent light brown seed coat that reveals a bent embryo in a peripheral position that surrounds a colourless perisperm. After examination under a binocular microscope, papillate ornamentation on the outer edge on the embryo was observed.

When comparing the morphological parameters of the different populations studied we found that Accession A had the highest length and width and correspondingly the heaviest seeds. On the other hand, accession C showed the lowest values for these same parameters (Table 1). The length/width ratio value was the lowest in accession A, which indicates that this seeds are more rounded than the others.

3.2. Seed germination

The germination percentages with alternating temperature (25/15 °C) reached values over 90% in all the samples (Table 2). No statistical differences were observed between compared accessions. The T_{50} values were low for accessions A and D, which reflects quite a rapid response. Accessions B and C germinated more slowly, with higher T_{50} values (Table 2). Significant differences in germination velocity were observed between these two groups, although all of them reached similar final germination percentages. These results reflect the fact that seed age, and thus storage time, did not seem to influence germination response (Table 1).

The germination percentages at constant temperature (20 °C) were significantly lower, and statistical differences were observed among accessions, with values from 4.0 to 31.8%. Higher percentages of germination at constant temperatures were obtained for the recently

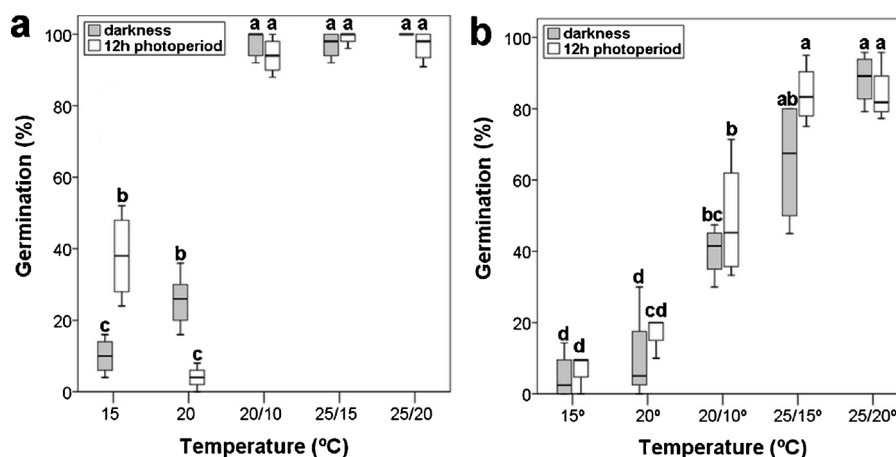


Fig. 1. Box plot of the germination percentages obtained under different temperature and light regimes for the studied *H. amplexicaulis* accessions. a. Accession A (2003); b. Accession C (2011). The same letters indicate homogeneous groups ($P < 0.05$).

collected seeds (Table 2). Germination velocity was similar in accessions A, B and C, and was significantly slower for accession D, which reached T_{50} after a considerable delay.

The RATG values showed a significant increase in the alternating temperature requirement related with seed age (Table 2). This index had lower values, which indicates stricter requirements for alternating temperatures in the oldest seeds collected 9 years before tests started.

The behaviour under a wider temperature range and different light conditions was also variable for accessions A and C (Fig. 1). The clear preference for alternating temperature was confirmed. Higher percentages were obtained at 20/10, 25/15 and 25/20 °C. In accession A, no statistical differences were found between these temperature regimes (Fig. 1a). However, in sample C when the average temperature of the applied alternating condition increased, the germination percentage was higher (Fig. 1b).

The effect of the illumination regime was significant only at constant temperatures in the seeds collected in 2003 (Fig. 1a). However, the observed responses were opposite at each temperature (15 and 20 °C) and the effect of light regime on germination percentage was, therefore, inconsistent; no clear requirement were detected. The RLG calculated for accessions A and C (Table 3) also demonstrate no clear preference for either condition as 0 corresponds to germination only under darkness, and 1 to germination only under the light condition.

The germination velocity, expressed by the T_{50} parameter, under different light regimes at the tested temperatures is reflected in Fig. 2. Statistical differences between light and darkness were found at temperatures 20, 20/10, 25/15 and 25/20 °C in accession A (Fig. 2a); faster germination was observed in the light tests with the exception of 25/20 °C. In accession C (Fig. 2b) the higher dispersion of the data determines no statistical differences between light regimes.

The results of the three way ANOVA showed a significant interaction between seed sample and temperature for both germination parameters, percentage and T_{50} ($F = 18.6$, $P < 0.001$ / $F = 9.4$, $P < 0.001$, respectively). The germination response related to temperature conditions was dependent on the accession studied (Figs. 1 and 2), especially for germination percentages, as indicated by the higher F value. The observed variation between the constant and alternating temperature regimes was strong or gradual depending on the tested

accession. No other strong interactions took place among the considered factors.

A stimulation of germination velocity was observed in the seeds exposed to the tested salt concentration. This halopriming effect was evident only at alternating temperatures (Table 4).

In order to check seed viability, the non-germinated seeds in the plates kept at a constant temperature were transferred to 25/15 °C, and total germination was reached after 2–3 days.

3.3. Climatic data measured in the ripening year and their correlation with seed parameters

Our study revealed a wide variation in the amount and distribution of rains between collecting years of the different accessions (Fig. 3). There were years with periods of absolute deficit in the growing season, versus each other with available water during almost the whole life cycle of this annual plant.

The specific climate data in autumn and winter, from October to March (period of maximum rain), in 2003 and 2013 corresponded to a semi-arid ombrotpe according to Ia values (Table 5). The same period in 2010 and 2011 could be classified as humid and dry-subhumid, respectively; the drought season was shorter and water deficit appeared and lasted only 3 to 4 months at the end of the plants' life cycle.

The correlation analysis led us to interpret that the fundamental climatic parameters, in the case of *H. amplexicaulis*, are those responsible of hydroperiod length. The cluster analysis result allowed the four considered periods to be grouped into two conglomerates (Fig. 4) based on different environmental factors. Cluster I corresponds to those years with a more extended aridity period, whereas cluster II to those with lower water deficit along the year. T12 to T3 and P10 to P3 had a significant effect on the separation of groups, especially the precipitation of March.

A very strong relationship between some morphological seed features (length, width and mass) and climatic parameters was observed (Table 6). Indeed, T, Tp, autumn and winter temperatures (T10, T11, T12 and T1) were the most directly connected parameters; the higher the temperatures, the larger seed size. A strong inverse correlation can be seen between seed characteristics and DF8, especially with weight.

Table 3

Relative light germination (RLG) values for accessions A and C at the different temperatures tested.

Accession	15 °C	20 °C	25/20 °C	20/10 °C	25/15 °C
A	0.8 ± 0.1a	0.1 ± 0.1c	0.5 ± 0.0b	0.5 ± 0.0b	0.5 ± 0.0b
C	0.7 ± 0.3ab	0.8 ± 0.1a	0.5 ± 0.0b	0.5 ± 0.1ab	0.6 ± 0.1ab

Within the same variable, the same letters indicate no significant differences.

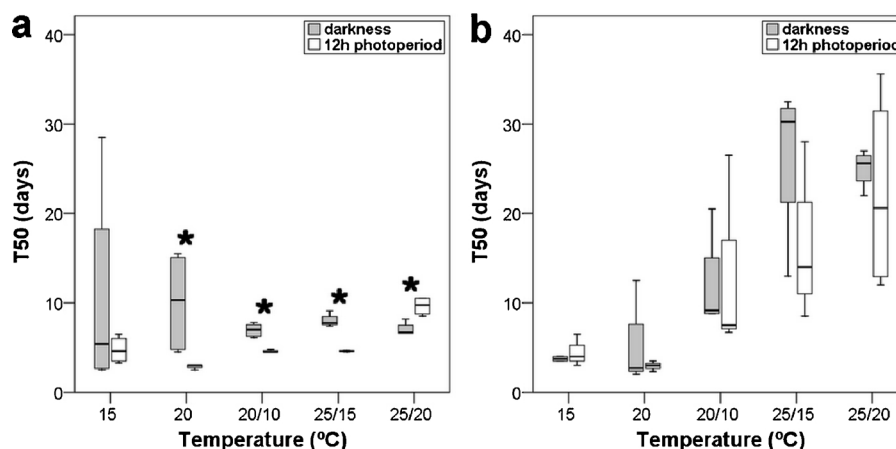


Fig. 2. Box plot of the T_{50} values obtained under different temperature and light regimes for the studied *H. amplexicaulis* accessions. a. Accession A (2003); b. Accession C (2011). Asterisks indicate significant differences between the light regimes used ($P < 0.05$).

Table 4
Effects of salt priming on the germination percentage and T_{50} .

	Accession	Temperature (°C)	Control	Priming	<i>P</i>
G %	B	25/15	93.0 ± 5.0	96.2 ± 2.5	0.307
		20	15.0 ± 4.1	14.9 ± 3.5	0.970
	C	25/15	95.0 ± 7.1	93.9 ± 0.5	0.779
		20	17.5 ± 5.0	27.1 ± 6.3	0.056
T_{50}	B	25/15	17.0 ± 3.7	4.3 ± 0.5	0.006
		20	2.9 ± 0.4	2.7 ± 0.4	0.518
	C	25/15	17.6 ± 4.6	3.3 ± 0.6	0.008
		20	3.0 ± 0.5	2.7 ± 0.4	0.484

P values of *t*-test are also given.

Concerning the germinative variables analysed, only germination velocity (T_{50}) at 25/15 °C was correlated with the climatic parameters considered. Table 6 shows a negative relationship of T_{50} at 25/15 °C with winter temperatures (T1, T2 and T3), water deficit during the period from germination to fruiting (DF3, DF6 and DF2-7) and the number of months with water deficit (NmDF). A positive correlation of precipitation (P10-3) and the aridity index (Ia10-3) from October to March, with time to 50% germination was observed (Table 6). No correlation between morphological and germinative parameters was detected.

4. Discussion

The species that inhabit in variable and unpredictable environments develop specific strategies to take advantage of available resources, and thereby ensure long-term population survival. In salt affected habitats, especially in dry environments, climatic conditions are driving factors for growing season length and plant responses (Guterman, 1994, 2000). In this sense, Debieu et al. (2013) established evidences for covariation among the life history traits of a taxon and the nature of the environmental gradient within its natural distribution range. This association has already been established between the inter-annual variation in precipitation and the seed density or species richness in diverse habitats (Santos et al., 2013).

The present research evidences the association between inter-annual variation of climatic conditions and seed features and responses in Mediterranean semi-arid salt marshes. The correlation analysis carried out (Table 6) led us to interpret that the fundamental climatic parameters in the case of annual plants inhabiting salt marshes, like *H. amplexicaulis*, are those responsible of hydroperiod length and, thus, the starting and success of the annual plant's life cycle (germination timing, seedling establishment, flowering and seed set). Otherwise, Foti et al.

(2012) suggested that the relative abundance of species is decisively dependent on the hydroperiod regime in wetlands. Our findings support and provide logical explanation to this assertion.

The variation observed in morphological seed parameters (size and weight) in *H. amplexicaulis* (Table 1) did not correlate with the different germination responses of the studied accessions. However, a clear relationship with some of the considered climatic parameters has been proved (Table 6). Our data, which are slightly above those indicated in the literature (Blanché and Molero, 1987), evidenced an increase in seed size and mass with higher temperatures in autumn and winter. This fact entails a longer vegetative period and thus great photosynthetic activity with the development of larger plants and many more resources to be allocated in the reproduction stage. The literature has provided significant reports about the influence of environmental pre- and post-fertilisation conditions on plant, seed set and even seed size (D'Antraccoli et al., 2018; Wei et al., 2018). Schimpf (1977) showed the association of larger seeds with drier environments, but Leishman et al. (2000) indicated that this association is quite limited. Vilellas and García (2013) evidenced a positive correlation between seed size and water stress, as a result of the combination between precipitation and temperature values.

Regarding the germination response of the tested accessions, significant differences were observed, even for the percentage and velocity, under the studied conditions. In accordance with diverse authors (Albert et al., 2002; Ferrer-Gallego et al., 2013; Santo, 2013), absolute preference for alternating temperature was found in this study (Fig. 1; Table 2). Conversely, De Martis et al. (1988), who studied the same population as Santo (2013), indicated high germination percentages at constant temperature (20 °C), although the results showed high variability depending on the sowing month, thus this data seems to be contradictory and not conclusive. Otherwise, a clear effect of light on seed germination has not yet been demonstrated for *H. amplexicaulis* (Figs. 1 and 2; Table 3) in contrast to the behaviour observed in other species of the same genus (Rasool et al., 2017). In the same way, the impermeability of the seed coat indicated by Albert et al. (2002) has not been confirmed either.

Our study links the preference by the alternating temperature regime to the fact that germination in periodically flooded habitats with irregular water incomes is usually controlled by daily temperature variation as a sensing mechanism, which indicates the end of the flooding period (Estrelles et al., 2015). This explanation is coherent with the field observations made by Tremblin (2000) about onset of germination.

Additionally, the increase of the proportion of seeds with requirement to alternating temperatures with age was evidenced by RATG values (Table 2). This response observed in old seeds favours the

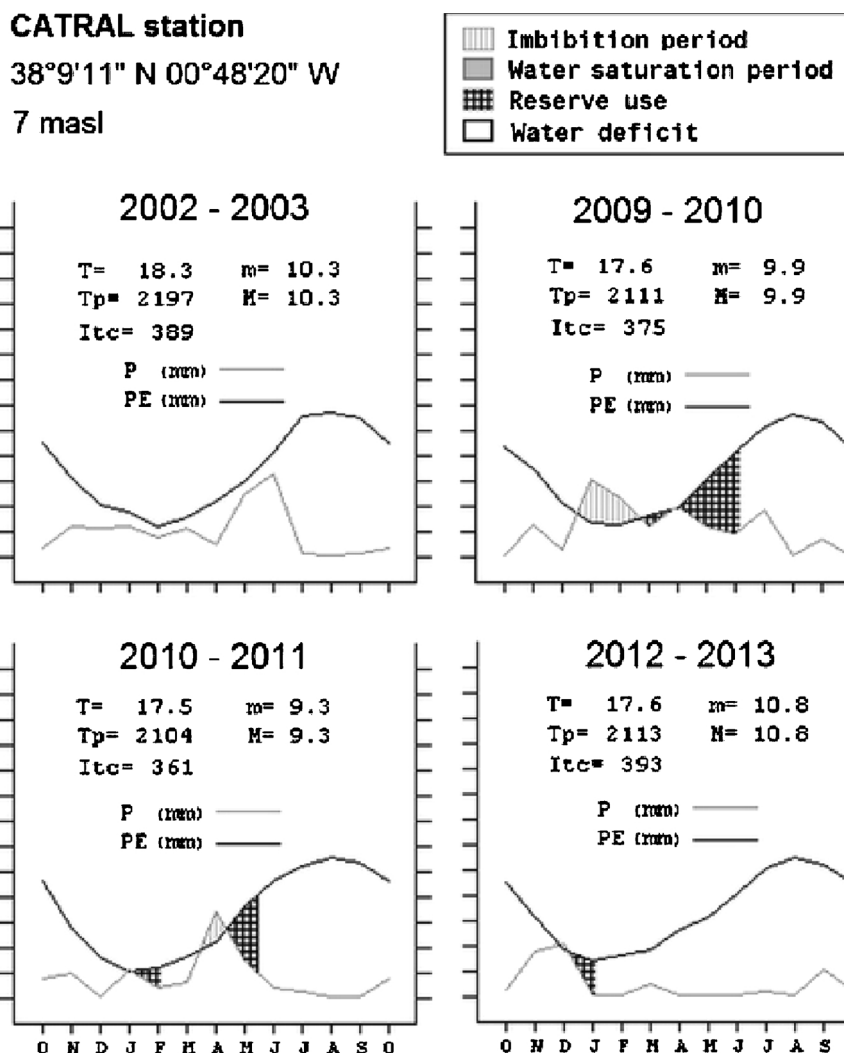


Fig. 3. Water balance graphs for the year that corresponds to the maternal environment for each studied accession (from October of the previous year of seed collecting, to September of the ripening year). T: yearly average temperature, m: yearly average temperature of minimum temperatures, M: yearly average temperature of maximum temperatures, Tp: Positive annual temperature, Itc: compensated thermicity index, P: annual precipitation and PE: potential evapotranspiration.

establishment of a long-term seed bank through seeds that are not immediately germinated and are more demanding by specific environmental conditions with age. This strategy seems to be crucial to ensure the survival of an annual plant population.

The major inter-accession differences in germination velocity, recorded in the conducted tests (Fig. 2; Table 2), permitted us to characterize two types of behaviour, a rapid response (represented by the accessions A and D) opposite to a slow germination (exhibited by the accessions B and C). These divergent responses could be described as opportunistic vs. bet-hedging strategies respectively, possibly

conditioned by the maternal plant environment, as we initially hypothesized.

From the cluster analysis results (Fig. 4), we also identified two climatic groups that corresponded to these two different germinative responses. Maternal environmental effect can be observed from the correlation analysis of the annual climatic data (Fig. 3; Table 5) with the specific germination behaviour for seeds collected during each particular year (Table 6). Specially, temperature and rainfall during the period that corresponds to the end of the winter and the start of spring had a huge impact on phenological timing and the population dynamics

Table 5
 Climate data recorded for the *Halopeplis amplexicaulis* population.

Period	T (°C)	P (mm)	Ps (mm)	P3 (mm)	P6 (mm)	Ic	Io	Ia 10-3	NmDF
1982-2012	18.3	291	76	20	14	15.4	1.3	0.8	–
2002-2003	18.3	236	123	9	2	17.2	1.1	0.5	7
2009-2010	17.6	285	79	39	36	16.2	1.4	0.9	2
2010-2011	17.5	182	104	68	5	16.8	0.9	0.6	5
2012-2013	17.6	111	0	0	2	15.5	0.5	0.3	7

The abbreviations used are detailed in Appendix A. T: Average temperature; P: Annual precipitation; Ps: Precipitation of the spring quarter; P3: Precipitation of March; P6: Precipitation of June; Ic: Continentality index; Io: Ombrothermic index; Ia: Aridity index; NmDF: Number of months with water deficit.

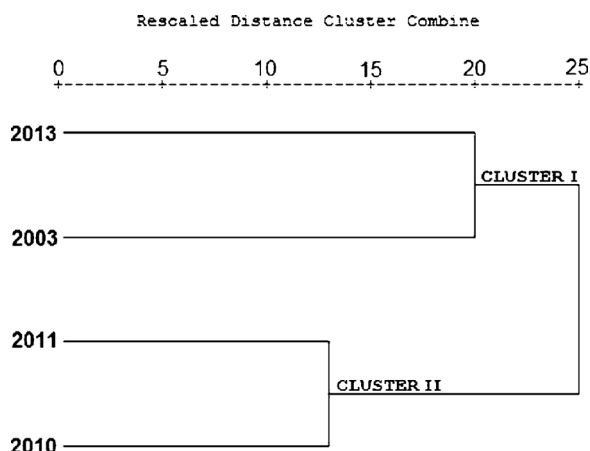


Fig. 4. Dendrogram for the cluster analysis conducted with 24 climatic variables (Ti and Pi). Dissimilarities are defined by Squared Euclidean distance and the combination of clusters is based on Ward's method.

of *H. amplexicaulis*. These statements justify the divergences on the cycle length and phenology, depending of the climatic conditions, previously indicated by diverse authors. For some Spanish populations, Suárez et al. (2007) indicated a cycle length of 3–4 months, depending on drought severity. Tremblin (2000) observed that the cycle was initiated in mid or late winter, and completed in 8–9 months for Algerian populations, depending on the rainfall period and temperature average. He also pointed out that seedlings began to appear on the edges of salt lagoons when temperature increased and the water level fell due to evaporation, and can continue several months until the centre of the small depressions become dry.

Water availability during the potential period for seedling establishment and fruit set is strongly correlated with the different germination strategies observed in the next generation. This behaviour suggested that the climate parameters in the precedent growing season could control the proportion of dormant and non-dormant seeds in the soil seed bank.

Previous research on this question proposed various key maternal factors in controlling the germination response and dormancy level (Guterman, 2000; Chen et al., 2014). Germain and Gilbert (2014) found a greater seed dormancy when the maternal plant was raised in a wet environment. This finding about reduced dormancy in drier environments is in accordance with the behaviour of *H. amplexicaulis*, and that might be associated with a survival advantage for this species.

Table 6

The Spearman coefficient (r) and associated P-values obtained in the correlation analysis of seed morphology and germinative response of *H. amplexicaulis* with those climatic variables (Cv showing a strong correlation (values > 0.8)).

Cv	Seed morphology						Germinative response		
	Mass		Length		Width		Cv	T ₅₀ 25/15 °C	
	r	P	r	P	r	P		r	P
T10	0.800	< 0.001	0.400	0.125	0.632	0.009	T1	-0.886	< 0.001
T11	1.000		0.800	< 0.001	0.949	< 0.001	T2	-0.862	< 0.001
T12	0.800	< 0.001	1.000		0.949	< 0.001	T3	-0.862	< 0.001
T1	0.400	0.125	0.800	< 0.001	0.632	0.009	P10-3	-0.886	< 0.001
T	0.800	< 0.001	1.000		0.949	< 0.001	Ia10-3	-0.862	< 0.001
m	-1.000		-0.800	< 0.001	-0.949	< 0.001	DF3	-0.921	< 0.001
TP	0.800	< 0.001	1.000		0.949	< 0.001	DF6	-0.921	< 0.001
RE10	-0.775	< 0.001	-0.775	< 0.001	-0.816	< 0.001	DF2-7	-0.862	< 0.001
DF10	0.800	< 0.001	0.400	0.125	0.632	0.009	R3	0.806	< 0.001
DF8	-1.000		-0.800	< 0.001	-0.949	< 0.001	NmDF	-0.806	< 0.001

The abbreviations used are detailed in Appendix A. T: Average temperature; m: Average minimum temperature of the coldest month; TP: Positive annual temperature; RE: Real evapotranspiration; DF: Soil water deficit; P: Precipitation; Ia: Aridity index; R: Soil water reserve; NmDF: Number of months with water deficit. Numbers following variable name indicate the corresponding month or period of months for each value (e.g. 1 correspond to January).

A possible explanation for the survival advantage achieved with this strategy could be the avoidance of sibling competition. Salt marshes are frequently refuges for less competitive species with diverse mechanisms of tolerance to high NaCl concentrations (Soriano et al., 2014), but sibling competition is frequent in this habitat, especially for annual plants. Higher resource availability (mainly water in arid territories) during the growth season lead to a higher seedling survival, higher plant density and therefore, higher seed set, with larger soil seed banks (Sotomayor and Gutiérrez, 2015). Consequently, a potentially higher number of individuals will compete for the resources available in the following year. Future studies should focus on the correlation of the exposed germinative behaviour with demography data in natural populations.

Köchy (2006) highlighted the essential role of a density regulation mechanism to avoid massive germination after heavy rainfall and, thus, excessive competition among plants, especially in semiarid environments. He signalled density-dependant germination as an adaptive behaviour in soil seed banks and an evolutionary stable strategy for annual species in Mediterranean sites. Delayed germination can act to avoid risk due to the combination of unpredictable abiotic conditions and variable density (Tielbörger and Valleriani, 2005; Gremer and Venable, 2014). The occasional presence of bet-hedging strategy in correlation with weather may be better understood under the wider vision provided by Volis and Bohrer (2013).

Our study proved the increase of seed germination velocity after salt exposition for the accessions with slow germination, even though the requirement of alternating temperature remains (Table 4). The natural osmopriming was evidenced as a post-dispersion control factor of seed germination, stimulating the response of 'slow seeds'. Natural priming may be promoted after successive dry periods when the soil seed bank is exposed to high salt concentrations during short rain events.

Our observations also support a heterogeneous seed bank model, where seed germination probability is variable according to the maternal and post-dispersal environment.

After considering this species' adaptations, we agree with Springthorpe and Penfield (2015) that future research should be conducted to analyse the potential climate change effect on the life history strategy of annual plants on natural populations, especially the seasonal displacement of rainfall. Indeed, changes in rainfall distribution could be more important than a decrease of total precipitation, and could have a key role in population dynamics of threatened species and in their locally extinction (Pugnaire et al., 2004).

5. Conclusions

The variation in the germination response in *H. amplexicaulis* is dependent on the maternal environment determining life cycle extent. Two different germination strategies, opportunistic vs. bet-hedging, were observed as a density regulation mechanism and soil seed bank control. The narrow adaptation of germination strategies to climatic conditions is a fundamental mechanism to avoid risks in unpredictable environments. Also, natural halopriming has been revealed as key factor controlling seed bank dynamics in halophytes. These aspects should be taken into account to better understand the reproductive biology of this species, and also when an effective germination protocol is needed to optimize germplasm *ex situ* collections and for planning nursery cultivation for recovery programmes. Our results are potentially applicable for the conservation and management of salt marshes ecosystems inhabited by the threatened annual species studied.

Acknowledgements

The authors would like to thank the Generalitat Valenciana's Wildlife Service and Vaersa's Biodiversity team in the province of Alicante, Juan Jiménez, Joan Pérez Botella, Roger Carchano and José L. Echevarrias for their support, guidance, notes on natural population evolution and seed harvesting at the El Hondo Natural Park.

This study was supported by European Union's EAFRD funds through the Operative Programme for the Valencian Community 2007-2013, regional measure 227 'Conservation and development of the Natura 2000 Network'.

Appendix A. List of abbreviations used to name the different climatic parameters considered in this study

- Dfi:** Soil water deficit for every month of the year.
Ia: Aridity index of Martonne [$P/(T + 10)$].
Iai: Aridity index of Martonne for every month of the year. [$P_i/(T_i + 10)$].
Ic: Continentality Index (yearly thermic interval). $Ic = T_{max} - T_{min}$. In degrees Celsius, the number expressing the range between the average temperatures of the warmest (T_{max}) and coldest (T_{min}) months of the year.
Io: Ombrothermic Index. $Io = (Pp/Tp) 10$. Ten times the quotient resulting value between the yearly positive precipitation in mm (Pp) and the yearly positive temperature (Tp) (see 'Pp' and 'Tp' above).
Ioi: Ombrothermic Index for every month of the year.
Ios1: Ombrothermic index of the warmest month of the summer quarter.
Ios2: Ombrothermic index of the warmest bimonth of the summer quarter. [$(Pps2/Tps2) 10$].
Ios3: Ombrothermic index of the three months of the summer quarter. [$(Pps3/Tps3) 10$].
Ios4: Ombrothermic index of the three months of the summer quarter and the previous month (May, June, July, Aug.). [$(Pps4/Tps4) 10$].
It: Thermicity Index. $It = (T + m + M) 10$. Ten times the sum of T (yearly average temperature), m (average minimum temperature of the coldest month of the year), M (average maximum temperature of the coldest month of the year). Coldest month of the year: the one that has the lowest monthly average temperature (T_{min}).
M: Average maximum temperature of the coldest month of the year.
m: Average minimum temperature of the coldest month of the year
NmDF: Number of months of with water deficit.
P: Yearly average precipitation in mm.
Pa: Total precipitation in mm of the autumn quarter (Sept., Oct., Nov., in the Northern Hemisphere).
Pi: Average precipitation any month of the year.
Pp: Yearly Positive Precipitation. In mm, total average precipitation

of those months whose average temperature is higher than 0°C .

Ps: Total precipitation in mm of the spring quarter (Mar., Apr. and May, in the Northern Hemisphere).

Psm: Total precipitation in mm of the summer quarter (June, July, Aug., in the Northern Hemisphere).

Pw: Total precipitation in mm of the winter quarter (Dec., Jan., Feb., in the Northern Hemisphere).

REi: Real evapotranspiration for every month of the year.

Ri: Soil water reserve for every month of the year.

T: Yearly average temperature in centigrade degrees (Celsius).

Ti: Average temperature for every month of the year.

References

- Aellen, P., 1967. *Halopeplis* Bunge ex Ung.-Sternb. In: In: Davis, P.H. (Ed.), Flora of Turkey and the East Aegean Islands Vol. 2. Edinburgh University Press, Edinburgh, pp. 319.
- Catálogo Valenciano de Especies de Flora Amenazadas. In: Aguilera, A., Fos, S., Laguna, E. (Eds.), Colección Biodiversidad 18. Conselleria de Medi Ambient, Aigua, Urbanisme i Habitatge, Generalitat Valenciana, Valencia.
- Albert, M.J., Iriondo, J.M., Pérez-García, F., 2002. Effects of temperature and pretreatments on seed germination of nine semiarid species from NE Spain. *Isr. J. Plant Sci.* 50, 103–112.
- Aud, F.F., Ferraz, I.D., 2012. Seed size influence on germination responses to light and temperature of seven pioneer tree species from the Central Amazon. *An. Acad. Bras. Cienc.* 84, 759–766.
- Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.
- Blanché, C., 1990. *Halopeplis* Bunge ex Ung.-Sternb. In: Castroviejo, S., Laínz, M., López-González, G., Montserrat, P., Muñoz-Garmendia, F., Paiva, J., Villar, L. (Eds.), Flora Ibérica II. Real Jardín Botánico-CSIC, Madrid, pp. 522–524.
- Blanché, C., Molero, J., 1987. The genus *Halopeplis* Ung.-Sternb. (Salicorniaceae) in the Iberian Peninsula. *Collect. Bot.* 17, 67–77.
- Chen, M., MacGregor, D.R., Dave, A., Florance, H., Moore, K., Paszkiewicz, K., Smirnov, N., Graham, I.A., Penfield, S., 2014. Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. *Proc. Natl. Acad. Sci. U. S. A.* 111, 18787–18792.
- D'Antraccoli, M., Roma-Marzio, F., Astuti, G., Bedini, G., Ciccarelli, D., Peruzzi, L., Carta, A., 2018. Variation of vegetative and reproductive traits in *Cistus laurifolius*: evidences of a response to contrasting habitat conditions. *Flora* 248, 22–27.
- De Martis, B., Loi, M.C., Sesselego, C., 1988. Prime indagini sull'autoecologia di *Halopeplis amplexicaulis*. *Thalassia Salentina* 18, 343–351.
- Debieu, M., Tang, C., Stich, B., Sikosek, T., Effgen, S., Josephs, E., Schmitt, J., Nordborg, M., Koornneef, M., de Meaux, J., 2013. Co-variation between seed dormancy, growth rate and flowering time changes with latitude in *Arabidopsis thaliana*. *PLoS One* 8, e61075. <https://doi.org/10.1371/journal.pone.0061075>.
- Del Cacho, M., Penuelas, J., Lloret, F., 2013. Reproductive output in Mediterranean shrubs under climate change experimentally induced by drought and warming. *Persp. Plant Ecol. Evol. Syst.* 15, 319–327.
- Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K., Willis, C.G., 2010. Germination, postgermination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Evol. Syst.* 41, 293–319.
- Estrelles, E., Biondi, E., Galiè, M., Mainardi, F., Hurtado, A., Soriano, P., 2015. Aridity level, rainfall pattern and soil features as key factors in germination strategies in salt-affected plant communities. *J. Arid Environ.* 117, 1–9.
- European Commission, 2013. Interpretation Manual of the European Union Habitats – EUR 28. DG Environment, Nature ENV B.3, Brussels.
- Fenner, M. (Ed.), 2000. Seeds: the Ecology of Regeneration in Plant Communities. CABI, Wallingford.
- Manual para la conservación de germoplasma y el cultivo de la flora valenciana amenazada. In: Ferrer-Gallego, P.P., Ferrando, I., Gago, C., Laguna, E. (Eds.), Manuales Técnicos Biodiversidad 3. Conselleria d'Infraestructures, Territori i Medi Ambient, Generalitat Valenciana, Valencia.
- Foti, R., del Jesus, M., Rinaldo, A., Rodríguez-Iturbe, I., 2012. Hydroperiod regime controls the organization of plant species in wetlands. *Proc. Natl. Acad. Sci. U. S. A.* 109, 19596–19600.
- Germain, R.M., Gilbert, B., 2014. Hidden responses to environmental variation: maternal effects reveal species niche dimensions. *Ecol. Lett.* 17, 662–669.
- Gremer, J.R., Venable, D.L., 2014. Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecol. Lett.* 17, 380–387.
- Guterman, Y., 1994. Strategies of seed dispersal and germination in plants inhabiting deserts. *Bot. Rev.* 60, 373–425.
- Guterman, Y., 2000. Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. *Plant Spec. Biol.* 15, 113–125.
- Iriondo, J.M., Albert, M.J., Giménez-Benavides, L., Domínguez, F., Escudero, L. (Eds.), 2009. Populations in Peril: Demographic Viability of Threatened Spanish Vascular Flora. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid.
- Jalas, J., Suominen, J., 1980. Atlas Florae Europaeae, 5. Chenopodiaceae to Basellaceae. Biological Society of Finland Vanamo, Helsinki.
- Khan, A., Rayner, G.D., 2003. Robustness to non-normality of common tests for the many-sample location problem. *J. Appl. Math. Decis. Sci.* 7, 187–206.
- Khan, M.A., Weber, D.J. (Eds.), 2008. Ecophysiology of High Salinity Tolerant Plants.

- Task for Vegetation Science 40. Springer, Dordrecht.
- Köchy, M., 2006. Opposite trends in life stages of annual plants caused by daily rainfall variability-interaction with climate change. In: Unal, Y., Kahya, C., Bari, D.D. (Eds.), Proceedings of the International Conference on Climate Change and the Middle East: Past, Present and Future. Istanbul Technical University, Istanbul, pp. 347–357.
- Laguna, E., Ballester, G., Deltoro, V.I., 2013. Plant micro-reserves (PMRs): origin and technical concepts. In: Kadis, C., Thanos, C., Laguna, E. (Eds.), Plant Micro-Reserves: from Theory to Practice. Experiences Gained from EU LIFE and Other Related Projects: PlantNet CY Project Beneficiaries. Utopia Publishing, Athens, pp. 4–12.
- Leishman, M.R., Wright, L.J., Moles, A.T., Westoby, M., 2000. The evolutionary ecology of seed size. In: Fenner, M. (Ed.), Seeds: the Ecology of Regeneration in Plant Communities. CABI, Wallingford, pp. 31–57.
- Long, R.L., Gorecki, M.J., Renton, M., Scott, J.K., Colville, L., Goggin, D.E., Commander, L.E., Westcott, D.A., Cherry, H., Finch-Savage, W.E., 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biol. Rev.* 90, 31–59.
- Mahmoud, A., El Sheikh, A.M., Baset, S.A., 1983. Germination of two halophytes: *Halopeplis perfoliata* and *Limonium axillare* from Saudi Arabia. *J. Arid Environ.* 6, 87–98.
- Milberg, P., Andersson, L., Thompson, K., 2000. Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Sci. Res.* 10, 99–104. <https://doi.org/10.1017/S0960258500000118>.
- Mudd, S.M., Fagherazzi, S., 2016. Salt marsh ecosystems: tidal flow, vegetation, and carbon dynamics. In: Johnson, E.A., Martin, Y.E. (Eds.), *A Biogeoscience Approach to Ecosystems*. Cambridge University Press, Cambridge, pp. 407–434.
- Noto, A.E., Shurin, J.B., 2017. Mean conditions predict salt marsh plant community diversity and stability better than environmental variability. *Oikos* 126, 1308–1318.
- Philippi, T., 1993a. Bet-hedging germination of desert annuals: beyond the first year. *Am. Nat.* 142, 474–487.
- Philippi, T., 1993b. Bet-hedging germination of desert annuals: variation among populations and maternal effects in *Lepidium lasiocarpum*. *Am. Nat.* 142, 488–507.
- Pugnaire, F.I., Armas, C., Valladares, F., 2004. Soil as a mediator in plant-plant interactions in a semi-arid community. *J. Veg. Sci.* 15, 85–92.
- Rasband, W.S., 1997–2014. *ImageJ*. Bethesda, Maryland, USA: U. S. National Institutes of Health. <http://imagej.nih.gov/ij/> (Accessed 10 January 2017).
- Rasool, S.G., Hameed, A., Khan, M.A., Gul, B., 2017. Seeds of *Halopeplis perfoliata* display plastic responses to various abiotic factors during germination. *Flora* 236, 76–83.
- Rivas-Martínez, S., Rivas-Sáenz, S., 1996–2009. *Worldwide Bioclimatic Classification System*. Spain: Phytosociological Research Center. <http://www.globalbioclimatics.org> (Accessed 16 November 2016).
- Santo, A., 2013. Seed Germination Requirements and Salt Stress Tolerance of Coastal Rare Species in Sardinia. Dottorato di ricerca in botanica ambientale ed applicata, Ciclo XXV. Università degli Studi di Cagliari, Cagliari.
- Santos, D.M., Silva, K.A., Albuquerque, U.P., Santos, J.M.F.F., Lopes, C.G.R., Araújo, E.L., 2013. Can spatial variation and inter-annual variation in precipitation explain the seed density and species richness of the germinable soil seed bank in a tropical dry forest in north-eastern Brazil? *Flora* 208, 445–452.
- Schimpf, D.J., 1977. Seed weight of *Amaranthus retroflexus* in relation to moisture and length of growing season. *Ecology* 58, 450–453.
- Soriano, P., Moruno, F., Boscaiu, M., Vicente, O., Hurtado, A., Llinares, J.V., Estrelles, E., 2014. Is salinity the main ecologic factor that shapes the distribution of two endemic Mediterranean plant species of the genus *Gypsophila*? *Plant Soil* 384, 363–379.
- Sotomayor, D.A., Gutiérrez, J.R., 2015. Seed bank of desert annual plants along an aridity gradient in the southern Atacama coastal desert. *J. Veg. Sci.* 26, 1148–1158.
- Springthorpe, V., Penfield, S., 2015. Flowering time and seed dormancy control use external coincidence to generate life history strategy. *eLife* 4, e05557. <https://doi.org/10.7554/eLife.05557>.
- Suárez, L.D., Serra, L., Rodríguez, M.T., Pérez-Botella, J., Cervantes, J., Sanz, G., Domínguez, F., Moreno, J.C., 2007. *Halopeplis amplexicaulis* (Vahl) Ces. Pass. et Gibelli (Chenopodiaceae). In: Bañares, A., Blanca, G., Güemes, J., Moreno, J.C., Ortiz, S. (Eds.), *Atlas y Libro Rojo de la Flora Vasculosa Amenazada de España*. Adenda 2006. Ministerio del Medio Ambiente, Dirección General para la Biodiversidad y Sociedad Española de Biología de la Conservación de Plantas, Madrid, pp. 72–73.
- Thanos, C.A., Doussi, M.A., 1995. Ecophysiology of seed germination in endemic labiates of Crete. *Isr. J. Plant Sci.* 43, 227–237.
- Tielbörger, K., Valleriani, A., 2005. Can seeds predict their future? Germination strategies of density-regulated desert annuals. *Oikos* 111, 235–244.
- Tremblin, G., Binet, P., 1982. Installation d'*Halopeplis amplexicaulis* (Vahl.) Ung. dans une sebkha algérienne. *Oecol. Plant.* 3, 373–379.
- Tremblin, G., Binet, P., 1984. Halophilie et résistance au sel chez *Halopeplis amplexicaulis* (Vahl.) Ung. *Oecol. Plant.* 5, 291–293.
- Tremblin, G., 2000. Comportement auto-écologique de *Halopeplis amplexicaulis*: plante pionnière des Sebkhas de l'Ouest algérien. *Rev. Sech.* 11, 109–116.
- UNEP, 1997. *World Atlas of Desertification*, 2nd edition. UNEP, London.
- Villellas, J., García, M.B., 2013. The role of the tolerance-fecundity trade-off in maintaining intraspecific seed trait variation in a widespread dimorphic herb. *Plant Biol.* 15, 899–909.
- Volis, S., Bohrer, G., 2013. Joint evolution of seed traits along an aridity gradient: seed size and dormancy are not two substitutable evolutionary traits in temporally heterogeneous environment. *New Phytol.* 197, 655–667.
- Wei, X., Liu, M., Wang, S., Jiang, M., 2018. Seed morphological traits and seed element concentrations of an endangered tree species displayed contrasting responses to waterlogging induced by extreme precipitation. *Flora* 246, 19–25.