

# Contrasting propagule dispersal and halophyte seed banks along the intertidal gradient

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**ABSTRACT:** Seed banks are key for resilience, secondary succession and restoration in ecosystems, especially in stressful environments. Even though salt marshes are an excellent ecosystem for studying seed bank dynamics along environmental stress gradients, few studies have analyzed these aspects on the entire intertidal gradient in the plant community as a whole. We recorded the density, species composition and distribution of sexual propagules, considering total number and only those propagules that contained seeds with an intact embryo, along the intertidal gradient in salt marshes (Ría Formosa, southwest Iberian Peninsula). The propagules mostly accumulated in the ecotone between the salt marshes and coastal dunes, acting as sink habitat. Beyond this, the highest densities of propagules were recorded in less stressful habitats. The most abundant propagules were usually found close to seed plant sources. Halophytes located along the intertidal gradient presented 3 different dispersal behaviors: short-distance dispersal, medium- to long-distance dispersal and a mix of both. A total of 12 of the 18 recorded propagules formed transient seed banks, reflected in lower species richness and diversity in the seed bank than in the established vegetation. Six taxa formed persistent seed banks, showing seeds with intact embryos throughout the year. Our results indicate that the persistent seed bank did not play an important role in sustaining the diversity of halophytes in the studied marshes. Their resilience mainly relied on seed dispersal, not on *in situ* germination from the seed bank.

**KEY WORDS:** Tidal salt marsh · Plant zonation · Dispersal · Persistent seed bank · Ría Formosa

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## 1. INTRODUCTION

Some plant species overcome periods of unfavorable environmental conditions by building up persistent seed banks (Walck et al. 2005). These seed banks may be key for resilience, secondary succession and regeneration in ecosystems since they increase local effective population size with enduring seeds that accumulate intergenerational genetic diversity (Honnay et al. 2008). Seed banks can also play an important role in the ecological restoration of plant communities (Bakker et al. 1996). Other plant

species have transient seed banks (<1 yr) as a result of rapid germination and low seed survival in the soil (Parsons 2012). Thus, knowing the relationships between the species composition of the seed bank and the standing vegetation is important for understanding plant population and community dynamics, and it may help conservationists to counter the effect of exotic invasive species and plan for ecological restoration (Hopfensperger 2007).

The physical and physiological characteristics of seeds, as well as environmental conditions, determine their behavior in the soil. Many plants coloniz-

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ing high-stress habitats, such as perennial halophytes and xerophytes, usually present transient seed banks containing small seeds with high and fast germination to take advantage of favorable conditions (Parsons 2012). In contrast, annual species and plants from very dynamic environments, such as seasonal wetlands, river margins and desert grasslands, build up persistent seed banks that make the ecosystems resilient to interannual variability (Goodson et al. 2001, Deil 2005).

At the same time, soil seed bank distributions show horizontal aggregation with seeds grouped in clusters in different plant communities and habitats for many ecosystems and species (Csontos 2007). Spatial segregation in species composition of seed banks should be particularly marked in those ecosystems with clear vegetation zonation patterns such as salt marshes (Noe & Zedler 2001), where dissemination of propagules by water (hydrochory) may play an important role in structuring plant communities (Nilsson et al. 2010). Tidal inundation in salt marshes controls abiotic factors such as flooding period, oxygenation and salinity, determining stress levels and resulting in marked environmental gradients along the intertidal frame (Ungar 1998, Castillo et al. 2000) which, together with biotic interactions, present clear vegetation zonation patterns (Engels et al. 2011). Thus, salt marshes are an ideal ecosystem model for studying propagule dispersal patterns and seed bank dynamics along environmental stress gradients. However, few studies have analyzed these aspects on the entire intertidal gradient across the plant community as a whole (e.g. Egan & Ungar 2000, Wolters & Bakker 2002), especially in salt marshes in a Mediterranean climate.

Previous studies have described the sedimentary environment, vegetation zonation and germination responses of halophytes to salinity along the intertidal gradient in Ría Formosa Natural Park (southwest Iberian Peninsula) (Contreras-Cruzado et al. 2017, Muñoz-Rodríguez et al. 2017). The detailed knowledge of these salt marshes and their halophytes provided a great opportunity to study the seed bank along an environmental stress gradient in relation to their plant communities. Here, our goal was to analyze seed bank dynamics along the intertidal gradient, and compare them to the structure of standing halophyte communities recorded for these marshes in a previous study (Contreras-Cruzado et al. 2017). With this aim, we recorded the density, species composition and distribution of sexual propagules (seed, fruits and accompanying structures), considering total number and only those propagules that contained

seeds with an embryo in salt marsh habitats along the whole intertidal gradient in a salt marsh area in Ría Formosa Natural Park. We hypothesized that most of the halophytes would show transient seed banks, as reported previously for many halophytes (Parsons 2012); therefore, the persistent seed bank of those species with a harder seed coat would show low diversity in comparison with standing vegetation. We also hypothesized that less stressful habitats along the intertidal gradient would show more diverse and larger seed banks than more stressful habitats suffering from anoxic or saltier conditions. This paper also discusses dispersal patterns and behaviors in the seed bank at the species and community level and their applications to salt marsh conservation. The relationships between seed bank and standing vegetation change between ecosystems and their successional stages (Hopfensperger 2007). In this context, knowledge of seed bank characteristics may help to manage against exotic species and plan ecological restoration efforts. Those seed banks that are diverse, including target species for ecosystem recovery, and that show similar composition to standing vegetation will be useful for ecological restoration (Bossuyt & Honnay 2008). Our study adds key information about the role of seed banks in the conservation of salt marshes, ecosystems threatened by sea level rises and anthropogenic disturbances.

## 2. MATERIALS AND METHODS

### 2.1. Study site

The dispersal of seeds and fruits was studied in the tidal salt marshes on the island of Tavira (37° 05' N, 7° 40' W) in the Ría Formosa mesotidal open estuary (Algarve, Portugal, Gulf of Cádiz, southwest Iberian Peninsula) that occupies an area of ca. 100 km<sup>2</sup>, of which 48 km<sup>2</sup> are covered by salt marshes (Teixeira & Alvim 1978) (Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/m616p051\\_supp.pdf](http://www.int-res.com/articles/suppl/m616p051_supp.pdf)). The area has a Mediterranean climate with Atlantic influence, with mild wet winters and hot dry summers. The semidiurnal tides inside the Ría Formosa lagoon vary from 0.5 m (neap tide) to 3.5 m (spring tide) with a mean range of 2.10 m and a mean spring tidal range of 2.97 m (Águas 1986). The Ría Formosa mesotidal lagoon is a system of marshes and tidal flats, separated from the Atlantic by a belt of sand dunes that extends for 55 km along the coast. Only 14% of the lagoon surface is permanently flooded and about 80% of

the lagoonal bottom emerges during spring low-water tides. Low and middle salt marshes are established on fine-grained alluvial sediments with sediment accretion rates ca. 8–9 mm yr<sup>-1</sup> (see Costa et al. 1996, Leitão et al. 1996, Andrade et al. 2004 and Contreras-Cruzado et al. 2017 for a detailed description of the hydrodynamics, physiography and vegetation of the study area). The study was carried out on a wide ungrazed intertidal gradient ca. 600 m long perpendicular to the tidal line, with a gentle slope where the typical pattern of vegetation zonation in the Gulf of Cádiz was clearly apparent. The habitats sampled (H1–H9) corresponded to the main vegetation bands parallel to

the tidal line, and were defined from the lowest to the highest distribution limits of the salt marshes: H1: bare intertidal mudflats; H2: *Spartina maritima* prairies; H3: low marshes dominated by *Sarcocornia perennis*; H4: middle marshes of *Halimione portulacoides* and *Sarcocornia fruticosa*; H5: *Inula crithmoides* formations; H6: high marshes of *Arthrocnemum macrostachyum*; H7: high marshes of *Limoniastrum monopetalum*; H8: sandy sediments colonized by *Salsola vermiculata*; H9: ecotone or transition zone between H8 and adjacent coastal dunes with abundant deposits of plant debris carried by the highest tides (Table 1, Fig. S2 in the Supplement).

Table 1. Studied salt marsh habitats, average distance to upper distribution limit of salt marshes (H9), characterization of their sedimentary environment (annual electric conductivity [Cond.], pH, organic matter content [OMC] and water content [WC]) and dominant standing halophyte vegetation along the intertidal gradient at Ría Formosa (southwest Iberian Peninsula). Sedimentary environment and species covers were recorded by Contreras-Cruzado et al. (2017). Values are mean  $\pm$  SE (n = 3–9)

Habitat	Distance to H9 (m)	Sedimentary environment	Dominant plant species
H1	597 $\pm$ 2	Cond. 16.2 $\pm$ 0.7 mS cm <sup>-1</sup> pH 7.5 $\pm$ 0.1 OMC 4 $\pm$ 0 % WC 27 $\pm$ 1 %	Bare intertidal mudflats
H2	594 $\pm$ 4	Cond. 25.1 $\pm$ 1.8 mS cm <sup>-1</sup> pH 7.2 $\pm$ 0.1 OMC 13 $\pm$ 2 % WC 44 $\pm$ 4 %	<i>Spartina maritima</i> (Curtis) Fernald
H3	590 $\pm$ 13	Cond. 21.4 $\pm$ 0.9 mS cm <sup>-1</sup> pH 7.0 $\pm$ 0.1 OMC 13 $\pm$ 1 % WC 51 $\pm$ 2 %	<i>Sarcocornia perennis</i> (Mill.) A.J. Scott
H4	500 $\pm$ 88	Cond. 27.4 $\pm$ 1.6 mS cm <sup>-1</sup> pH 7.0 $\pm$ 0.1 OMC 22 $\pm$ 1 % WC 60 $\pm$ 2 %	<i>Halimione portulacoides</i> Aelen and <i>Sarcocornia fruticosa</i> (L.) A.J. Scott
H5	403 $\pm$ 43	Cond. 15.5 $\pm$ 1.0 mS cm <sup>-1</sup> pH 7.5 $\pm$ 0.0 OMC 8 $\pm$ 0 % WC 24 $\pm$ 1 %	<i>Inula crithmoides</i> L.
H6	247 $\pm$ 51	Cond. 18.0 $\pm$ 1.9 mS cm <sup>-1</sup> pH 8.0 $\pm$ 0.1 OMC 4 $\pm$ 0 % WC 13 $\pm$ 1 %	<i>Arthrocnemum macrostachyum</i> (Moric.) K. Koch
H7	93 $\pm$ 6	Cond. 11.7 $\pm$ 1.6 mS cm <sup>-1</sup> pH 8.7 $\pm$ 0.1 OMC 3 $\pm$ 1 % WC 2 $\pm$ 0 %	<i>Limoniastrum monopetalum</i> (L.) Boiss
H8	41 $\pm$ 14	Cond. 2.0 $\pm$ 0.7 mS cm <sup>-1</sup> pH 8.7 $\pm$ 0.1 OMC 1 $\pm$ 0 % WC 1 $\pm$ 0 %	<i>Salsola vermiculata</i> L.
H9	0 $\pm$ 0	Cond. 0.2 $\pm$ 0.1 mS cm <sup>-1</sup> pH 8.6 $\pm$ 0.1 OMC 2 $\pm$ 0 % WC 1 $\pm$ 0 %	<i>Salsola vermiculata</i> L. and invasive <i>Carpobrotus edulis</i> (L.) N.E. Br.

## 2.2. Seed bank sampling and analysis

Soil samples were collected using stainless steel cores (50 mm diameter, 50 mm height) during low tides at each of the 9 above-mentioned habitats (Table 1) in March, May, July, October and December 2012 (at each date  $n = 9$  soil samples at each habitat H2–H9;  $n = 12$  at H1 due to its low vegetation cover), resulting in a total of 420 soil samples. Previous studies have reported that most of the seeds accumulate in the first 50 mm of salt marsh sediments (Coteff & Van Auken 2006, Zepeda et al. 2014). After collecting, soil samples were stored in polyethylene bags and stored at  $-20^{\circ}\text{C}$  until analysis in the laboratory.

Methods based on germination to record the number of propagules were discarded as they are less precise than direct extraction, because many seeds can remain dormant (Gross 1990, Brown 1992). Soil samples were weighed ( $107 \pm 1$  g), and 6 subsamples of 5.0 g (corresponding to ca. 30 % of each sample) were chosen randomly from each soil core, following Bigwood & Inouye (1988), who stated that the precision of seed-number estimates is improved by subsampling large whole units with very small subunits. Each seed and fruit and the accompanying structures found in cores (hereafter, propagules) was carefully extracted from all subsamples, avoiding loss and breakage. Fine soil particles were eliminated by placing each subsample in a solution of sodium polyphosphate ( $50 \text{ g l}^{-1}$ ) and sodium bicarbonate ( $25 \text{ g l}^{-1}$ ) (Dorado et al. 1999), and by sieving them with 0.4 mm mesh spacing (Malone 1967). The material that remained in the filter (sand, gravel, shells, wrack and propagules) was then placed on a white plastic surface marked by  $1 \times 1$  cm squares; each plot was examined under a magnifying glass and the propagules were recorded and extracted. The propagules were identified to genus or species level using the propagule collection of our University of Huelva research group, and by referring to descriptions in Valdés et al. (1987) and Castroviejo (2015). The presence of seeds with intact embryo was explored for each sexual propagule through dissection under a magnifying glass, except for the seeds of the holoparasite *Cistanche phelypaea*, *Frankenia laevis* and the genus *Spergularia* that were too small to distinguish their embryo. Then, the densities of the propagules for each sample point at each time were calculated by calculating their density in each subsample (no. propagules per subsample weight) and their mean for the sample (propagules per sample weight), and then estimating the number of propagules  $\text{m}^{-2}$  sample point. The densities of the propagules for each

habitat and date were calculated as the mean of the 9–12 soil samples  $\text{habitat}^{-1}$ . The seed bank structure (taking into account only those propagules containing seeds with an embryo recorded throughout the year) and vegetation absolute cover (recorded at the same sample points as the seed bank by Contreras-Cruzado et al. 2017 a using visual method; Bergstedt et al. 2009; in squares of  $10 \times 10$  m,  $n = 3$  plots  $\text{habitat}^{-1}$ ) at each marsh habitat (Table 1) were calculated in terms of total number of species ( $S$ ), biological diversity following the Shannon-Wiener index ( $H'$ ) (Shannon & Weaver 1949) and evenness ( $J'$ ) (Simpson 1949). A species was considered to form a persistent seed bank when its seeds with embryo were recorded during most of the year. Dispersal patterns were characterized following the relationship between the spatial pattern of the seed bank and the standing distribution of each taxon. A taxon was classified as a short-distance disperser when its propagule density was related to its standing cover and as medium- to long-distance disperser when its propagule density was independent of its cover.

## 2.3. Statistical analysis

Statistics were carried out with STATISTICA 8.0 (Statsoft), applying a significance level of  $\alpha \leq 0.05$ . Deviation was calculated as standard error of the mean (SE). Normality of the data series was tested using the Shapiro-Wilk test, and homogeneity of variance by Levene's test. When homogeneity of variance and normality were not achieved, data were transformed using the following functions  $1/(x+1)$ ,  $\log(x+1)$ ,  $\arcsin(x)$  and  $\sqrt{x}$ , and as the data were still not normal in their distribution or homogeneous in their variances, propagule densities and diversity indices were compared between habitats and sampling dates using the non-parametric Kruskal-Wallis  $H$ -test and Mann-Whitney  $U$ -test as post hoc tests. The relationship between the cover of halophyte species (recorded previously by Contreras-Cruzado et al. 2017) and propagule densities ( $n = 420$  samples) and between the diversity indices for the seed bank and vegetation ( $n = 28$  samples) were explored along the intertidal gradient using Spearman's correlation coefficient ( $\rho$ ).

## 3. RESULTS

A total of 26 different sexual propagules (fruits and seeds) were identified; 1.8 % of propagules could not be identified. Eight types of propagules (0.9 % of

propagules) corresponded to psammophytes colonizing adjacent coastal dunes, which accumulated mainly in H9 (Kruskal-Wallis test,  $H_{8,420} = 16.68$ ,  $p < 0.05$ ) without showing any seasonal dispersal pattern ( $H_{5,420} = 9.27$ ,  $p = 0.099$ ).

The total propagule density of halophytes was  $10977 \pm 1259$  propagule  $m^{-2}$  belonging to 18 identified halophyte taxa (Table 2, Fig. 1). The seeds of the genus *Spergularia* (with 2 species in the studied area: *S. marina* and *S. heldreichii*), *Suaeda* (*S. albescens*, *S. spicata* and *S. vera*) and *Sarcocornia* (*S. perennis* and *S. fruticosa*) and the fruits of *Limonium ferulaceum* and *L. diffusum* could not be identified to species level.

The lowest densities of halophyte propagules were recorded at H1, H7 and H8. The ecotone between marshes and dunes (H9) accumulated many more propagules than any other habitat; 9 of the 18 identified taxa in the seed bank were more abundant at H9 than in other habitats ( $H_{8,420} = 107.07$ ,  $p < 0.0001$ ) (Fig. 2A). H3, H4 and H9 tended to accumulate more seeds with embryo than other habitats, however, no significant differences were recorded (Kruskal-Wallis test,  $p > 0.05$ ) (Fig. 2C). Halophyte propagules tended to be more abundant in December and less in May–July ( $H_{4,420} = 15.80$ ,  $p < 0.005$ ) (Fig. 2B). Seeds with embryo tended to be more abundant in the seed bank in October and less in May (Kruskal-Wallis test,  $p > 0.05$ ) (Fig. 2D).

The propagules of 7 halophyte species (*Inula crithmoides*, *Frankenia laevis*, *Polygonum equisetiforme*, *Puccinellia convoluta*, *Atriplex halimus* [not present in the habitats studied but colonizing nearby high marshes], *Limonium narbonense* and *Spartina maritima*) were very scarce in the sediments, being recorded in fewer than 4 soil samples. Two-thirds of the collected fruits of *I. crithmoides*, both sampled fruits of *F. laevis*, half the achenes of *P. equisetiforme* and the only sampled fruit of *P. convoluta* and *A. halimus* all contained seeds with embryo. The only propagule of *L. narbonense* and *S. maritima* sampled had no seed.

*L. ferulaceum* plus *L. diffusum*, *Salsola vermiculata*, *Halimione portulacoides* and *Salicornia ramosissima* showed less than 20 propagules in all soil samples, always with densities lower than 600 propagules  $m^{-2}$ . All recorded propagules of *L. ferulaceum* plus *L. diffusum* and *S. vermiculata* had no seeds and their highest densities were sampled at H9 and H6 (Kruskal-Wallis test,  $p < 0.005$ ) (Fig. 3A,C). The propagules of *H. portulacoides* were more abundant at H4 and H6 ( $H_{8,420} = 18.65$ ,  $p < 0.05$ ), where this species' cover was higher ( $\rho = +0.183$ ,  $p < 0.05$ )

(Fig. 3E); only 3.8 % of its seeds had embryo at H6 in December. Most of the seeds of *S. ramosissima* had embryo (87.8 %), being recorded only at H2 and H3 ( $H_{8,420} = 43.01$ ,  $p < 0.0001$ ) (Fig. 3G). None of these halophytes showed seasonal differences in their propagule distribution (Kruskal-Wallis test,  $p > 0.05$ ) (Fig. 3B,D,F,H).

The most abundant taxa in the seed bank (with densities higher than 600 propagules  $m^{-2}$  in some marsh habitats) were *Suaeda* sp., *Limoniastrum monopetalum*, *Arthrocnemum macrostachyum*, *Limonium algarvense*, *Sarcocornia* sp., *Spergularia* sp. and *Cistanche phelypaea*. Most of the seeds of *Suaeda* sp. were hollow (99.8 %) and they were mostly accumulated at H9, H5 and H4 ( $H_{8,420} = 69.79$ ,  $p < 0.0001$ ). The few seeds with embryo in the seed bank were only at H6 and H9 (Fig. 4A). The seeds of *Suaeda* sp. were more abundant in patches with higher cover of *S. albescens* ( $\rho = +0.279$ ,  $p < 0.05$ ), and those with higher sum cover of the 3 species of *Suaeda* ( $\rho = +0.164$ ,  $p < 0.05$ ), and were independent of the cover of *S. spicata* and *S. vera*. Maximum densities of propagules of *Suaeda* sp. were recorded in December, and minimum in May ( $H_{4,420} = 16.18$ ,  $p < 0.005$ ) (Fig. 4B). The spicules of *L. monopetalum* contained seeds with embryo in 3.4 % of cases (recorded only at H9). The highest spicule densities were recorded at H9 ( $H_{8,420} = 132.86$ ,  $p < 0.0001$ ) (Fig. 4C). Disregarding H9, the density of spicules was higher at H6 and H7 with higher cover of *L. monopetalum* ( $\rho = +0.188$ ,  $p < 0.05$ ), without showing any seasonal differences ( $H_{4,420} = 4.36$ ,  $p = 0.360$ ) (Fig. 4D). *A. macrostachyum* presented 15.9 % of seeds with intact embryo. The highest seed densities were recorded in H9 and H6 ( $H_{8,420} = 63.18$ ,  $p < 0.0001$ ) (Fig. 4E), being more abundant where cover from *A. macrostachyum* was higher ( $\rho = +0.142$ ,  $p < 0.05$ ) without showing any seasonal pattern ( $H_{4,420} = 7.70$ ,  $p = 0.3195$ ) (Fig. 4F). *L. algarvense* showed 3.2 % of its spicules with seed with embryo, being more abundant at H9 and H6 ( $H_{8,420} = 80.78$ ,  $p < 0.0001$ ) (Fig. 4G) where its cover was higher ( $\rho = +0.119$ ,  $p < 0.05$ ), without showing any seasonal pattern ( $H_{4,420} = 8.41$ ,  $p = 0.078$ ) (Fig. 4H). *Sarcocornia* sp. had 9.3 % of seeds with embryo. The seeds of *Sarcocornia* were more abundant at H3 ( $H_{8,420} = 102.78$ ,  $p < 0.0001$ ) (Fig. 4I), coinciding with maximum cover from *S. perennis* ( $\rho = +0.284$ ,  $p < 0.05$ ) and of both *Sarcocornia* species ( $\rho = +0.385$ ,  $p < 0.05$ ), being independent of cover from *S. fruticosa*. The total number of *Sarcocornia* seeds was maximum in December and minimum in May ( $H_{4,420} = 20.89$ ,  $p < 0.0005$ ). *Sarcocornia* seeds with intact embryo were recorded during the whole study period (Fig. 4J). The seeds of *Spergularia* sp.



Table 2. Families and scientific names, species habit, colonized habitats and seed, fruit and propagule characteristics for plant species recorded in the seed bank of salt marshes distributed along the intertidal gradient at Ría Formosa (southwest Iberian Peninsula). Data sources: A. F. Muñoz-Rodríguez (pers. obs.), Castroviejo (1986–2015), Valdés et al. (1987) and Fernández-Illescas et al. (2010). -: taxon not found in the study area growing as adult plants

Species	Species habit	Habitats	Fruit type	Accessory structures when dispersed	Propagule	Propagule cover	Endosperm	Fruit or seed size (mm)	Months of flowering/ fruiting
<b>Asteraceae</b>									
<i>Inula crithmoides</i> L.	Chamaephyte	5 > 4	Cypsela	None	Cypsela with vilano	Coriaceous	Endosperm	1.5–2.3	VII–XI
<b>Caryophyllaceae</b>									
<i>Spergularia</i> sp.									
<i>S. heldreichii</i> Foucaud	Therophyte	7 > 8 > 9	Capsule	None	Seed	Coriaceous	Endosperm	0.3–0.6	IV–VI
<i>S. marina</i> (L.) Besser	Therophyte/ chamaephyte	4	Capsule	None	Seed	Coriaceous	Endosperm	0.5–0.8	III–VIII
<b>Chenopodiaceae</b>									
<i>Arthrocnemum macrostachyum</i> (Moric.) K. Koch	Phanerophyte	6 > 5 > 7 > 4	Utricle	Ephemeral perianth	Seed	Coriaceous	Perisperm	ca. 1.3	IV–VI
<i>Atriplex halimus</i> L.	Phanerophyte	–	Utricle	Bracts	Accessory fruit	Membranous	Perisperm	ca. 8	VI–XII
<i>Halimione portulacoides</i> Aelen	Chamaephyte/ phanerophyte	4 > 3 > 6 > 5 > 2	Utricle	Persistent bracts	Accessory fruit	Membranous	Perisperm	2–4	VII–XI
<i>Salicornia ramosissima</i> J. Woods	Therophyte	2	Utricle	Ephemeral perianth	Seed	Membranous	No	1.1–1.4	V–XI
<i>Salsola vermiculata</i> L.	Phanerophyte	9 > 8 > 7	Utricle	Persistent perianth with wings	Accessory fruit	Membranous	Perisperm	1.8–2.0	VI–XI
<i>Sarcocornia</i> sp.									
<i>S. fruticosa</i> (L.) A.J. Scott	Chamaephyte/ phanerophyte	4 > 6 > 5 > 2 > 3	Utricle	Ephemeral perianth	Seed	Membranous	No	0.6–1.5	IX–XII
<i>S. perennis</i> (Mill.) A.J. Scott	Chamaephyte	3 > 2 > 4	Utricle	Ephemeral perianth	Seed	Membranous	No	0.6–1.5	IX–XII
<i>Suaeda</i> sp.									
<i>S. albens</i> Lázaro Ibiza	Therophyte	5 > 3 > 4 > 6 > 7 > 2	Utricle	Ephemeral perianth	Seed	Coriaceous	No	1.0–2.0	I–IX
<i>S. spicata</i> (Willd.) Moq.	Therophyte	7 > 6 > 8	Utricle	Ephemeral perianth	Seed	Coriaceous	No	0.6.1.2	VI–X
<i>S. vera</i> Forsk.	Phanerophyte	6 > 7 > 5	Utricle	Ephemeral perianth	Seed	Coriaceous	No	0.8–2.0	III–XI
<b>Frankeniaceae</b>									
<i>Frankenia laevis</i> L.	Chamaephyte	7 > 8 > 9	Capsule	None	Seed	Coriaceous	Endosperm	<1	IV–XI
<b>Plumbaginaceae</b>									
<i>Limoniastrum monopetalum</i> (L.) Boiss	Phanerophyte	7 > 6 > 5 > 8 > 9 > 4	Utricle	Persistent bracts	Spikelet	Coriaceous	Endosperm	ca. 10	IV–XI
<i>Limonium algarvense</i> Erben	Chamaephyte	5 > 6 > 7 > 8 > 9	Utricle	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	ca. 5	V–VIII

Table 2 (continued)

Species	Species habit	Habitats	Fruit type	Accessory structures when dispersed	Propagule	Propagule cover	Endosperm	Fruit or seed size (mm)	Months of flowering/ fruiting
<i>Limonium ferulaceum</i> plus <i>L. diffusum</i>									
<i>L. ferulaceum</i> (L.) Chaz.	Chamaephyte	6 > 5 > 4 > 7 > 8	Utricle	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	ca. 5	VI–VIII
<i>L. diffusum</i> (Pourr.) Kuntze	Chamaephyte	6 > 5 > 4 > 7 > 8	Utricle	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	3–7	VI–VIII
<i>Limonium narbonne</i> Mill.	Chamaephyte	4 > 5 > 2 > 6	Utricle	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	ca. 6	VII–IX
<b>Poaceae</b>									
<i>Puccinellia convoluta</i> (Hornem.) Fourr.	Hemicryptophyte	4 > 5 > 2 > 6 > 7	Caryopsis	Persistent glumelles	Spikelet	Herbaceous	Endosperm	ca. 5	IV–VII
<i>Spartina maritima</i> (Curtis) Fernald	Geophyte	2 > 3 > 4	Caryopsis	Persistent glumes and glumelles	Spikelet	Herbaceous	Endosperm	10.0–12.5	V–VII
<b>Polygonaceae</b>									
<i>Polygonum equisetiforme</i> Sibth. & Sm.	Geophyte	8 > 9 > 7	Aquenio	None	Seed	Coriaceous	Endosperm	2–5	IV–XII
<b>Orobanchaceae</b>									
<i>Cistanche phelypaea</i> (L.) Cout.	Geophyte	5	Capsule	None	Seed	Coriaceous	Endosperm	ca. 0.5	III–X

were more abundant at H8 and H9 ( $H_{8,420} = 18.89$ ,  $p < 0.05$ ) (Fig. 4K), coinciding with higher cover from *S. marina* ( $p = +0.097$ ,  $p < 0.05$ ), *S. heldreichii* ( $p = 0.173$ ,  $p < 0.05$ ) and their combination ( $p = +0.204$ ,  $p < 0.05$ ). No seasonal pattern was recorded in the *Spergularia* seed bank ( $H_{4,420} = 4.72$ ,  $p = 0.318$ ) (Fig. 4L). The seeds of *C. phelypaea* were more abundant at H3 and H4 ( $H_{8,420} = 21.29$ ,  $p < 0.01$ ) (Fig. 4M), coinciding with its higher cover ( $p = +0.184$ ,  $p < 0.05$ ), and they were present only during October–December ( $H_{4,420} = 29.53$ ,  $p < 0.0001$ ) (Fig. 4N).

The highest *S* for the standing vegetation (13 species) was recorded at upper marsh habitats (H7–H9) ( $H_{8,28} = 25.06$ ,  $p < 0.005$ ), whereas *S* for the seed bank (seeds with embryo) was always lower than 3.5 species without showing any significant differences between habitats (Kruskal-Wallis *H*-test,  $p > 0.05$ ) (Fig. 5A). *H'* and *J'* for the standing vegetation were maximum at H6 and H7 (ca. 1.59 and 0.69, respectively), decreasing towards both marsh edges (*H'*:  $H_{8,28} = 24.43$ ,  $p < 0.005$ ; *J'*:  $H_{8,28} = 19.56$ ,  $p < 0.05$ ). In contrast, *H'* for the seed bank was always lower than 1.1, and *J'* varied between 0.26 and 0.90, without showing any significant differences between habitats (Kruskal-Wallis *H*-test,  $p > 0.05$ ) due to high heterogeneity in the seed bank. Even so, *H'* in the seed bank tended to be higher at H3 and H9 than at the other habitats (Fig. 5B,C).

Three distinct dispersal patterns were distinguished following above-mentioned relationships between the spatial distribution of propagules and standing halophyte cover along the studied intertidal gradient (see Table 1): (1) *Sarcocornia* sp., *Cistanche phelypaea*, *H. portulacoides* and *S. ramosissima* showed a short-distance dispersal since most of their propagules were recorded at the habitats of the seed mother plants; (2) *S. maritima*, *S. vermiculata* and *I. crithmoides* shared a medium-to long-distance dispersal with most of their propagules recorded 100s of m away from the mother plants; and (3) the rest of taxa presented a mixed behavior since their propagules were recorded both at the mother plant habitats and at other



Fig. 1. Propagules described in Table 2. (A) Cypselas with vilano of *Inula crithmoides*; (B) seeds of *Spergularia heldreichii*; (C) seeds of *Arthrocnemum macrostachyum*; (D) utricles and accessory fruits of *Atriplex halimus*; (E) accessory fruits of *Halimione portulacoides*; (F) seeds of *Salicornia ramosissima*; (G) utricles and accessory fruits of *Salsola vermiculata*; (H) seeds of *Sarcocornia perennis*; (I) seeds of *Suaeda albescens*; (J) fruiting calyx and capsule of *Frankenia laevis*; (K) spikelets of *Limonistrum monopetalum*; (L) spikelets of *Limonium algarvense*; (M) spikelets *Limonium ferulaceum*; (N) fruiting calyces of *Limonium narbonense*; (O) florets *Puccinellia convoluta*; (P) spikelets of *Spartina maritima*; (Q) seeds of *Polygonum equisetiforme*; and (R) seeds of *Cistanche phelypaea*

habitats 100s of m away where mother plants were absent from the standing vegetation (Figs. 3 & 4).

#### 4. DISCUSSION

Most of the propagules were collected at the ecotone between the salt marshes and coastal dunes colonized only by 3 halophytes (*Salsola vermiculata*, *Frankenia laevis* and *Limonium algarvense*) (Contreras-Cruzado et al. 2017). The propagules would have been transported to this ecotone by the highest tides and by coastal winds in the case of

those psammophyte propagules originating in the dunes. In contrast, the adjacent sandy sediments colonized by *S. vermiculata*, together with the unvegetated mudflats at the lower distribution limit of the marshes, were the habitats with the lowest propagule density. In line with our study, Huiskes et al. (1995) and Wolters & Bakker (2002) recorded higher propagule densities at upper levels of the marsh than at the habitats colonized by seed plants (source habitats). Thus, the ecotone between salt marshes and adjacent coastal dunes was acting as a sink habitat as it received most of the seeds of many halophytes that were not getting established there. Studies in fresh-



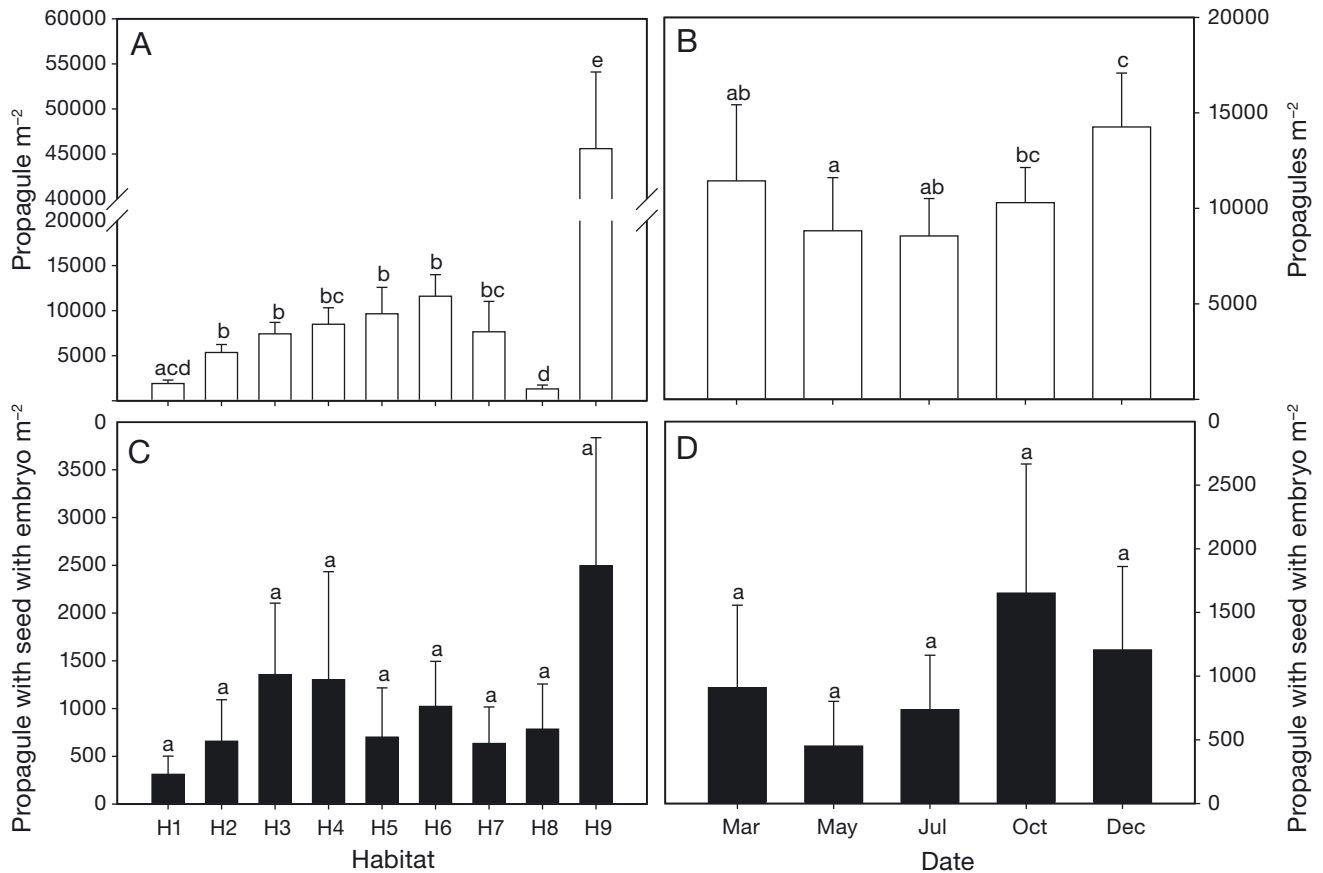


Fig. 2. Density of (A,B) total propagules and (C,D) those that contain seeds with embryo of halophytes in the sediments at 9 salt marsh habitats (H1–H9) along the intertidal gradient (A,C) and in 5 months (March, May, July, October and December) (B,D) at Ría Formosa (southwest Iberian Peninsula). Different lowercase letters indicate significant differences between habitats or dates (Mann-Whitney *U*-test,  $p < 0.05$ ). Habitats: H1: bare mudflats; H2: *Spartina maritima* prairies; H3: low marshes of *Sarcocornia perennis*; H4: middle marshes of *Halimione portulacoides* and *Sarcocornia fruticosa*; H5: *Inula crithmoides* formations; H6: high marshes of *Arthrocnemum macrostachyum*; H7: high marshes of *Limoniastrum monopetalum*; H8: sandy sediments colonized by *Salsola vermiculata* L.; H9: ecotone between marshes and coastal dunes. Values are mean  $\pm$  SE ( $n = 45$  samples habitat<sup>-1</sup> H2–H9 and  $n = 60$  in H1;  $n = 84$  samples date<sup>-1</sup>)

water marshes reported higher seed densities in less flooded habitats (Parker & Leck 1985, Hopfensperger & Engelhardt 2008). This source–sink dynamic established along the intertidal gradient reflected the capacity of most of the halophytes for medium- to long-distance dispersal by tides. In fact, diversity in the seed bank tended to be higher in *Sarcocornia perennis* marshes and at the upper ecotone than in other habitats, coinciding with mean high water and highest astronomical tide, respectively.

Beyond the role of the upper ecotone as a sink habitat for propagules, the most abundant propagules were usually found close to seed plant sources as reflected by correlation analyses between species cover and their seed bank density, leading to a highly heterogeneous seed bank composition along the intertidal gradient. Thus, 7 of the 9 habitats had their own unique seed bank composition. Studies of salt

marshes have also recorded heterogeneous seed bank composition dependent on halophyte cover (Rand 2000, Noe & Zedler 2001), whereas others found weak correlation between the above-ground vegetation and seed bank composition (Egan & Ungar 2000). According to our hypothesis, less stressful habitats dominated by *Inula crithmoides* (H5) and *Arthrocnemum macrostachyum* (H6) presenting well-drained and humid sediments (water contents between 10 and 25%) with medium salinities (conductivities between 10 and 20 mS cm<sup>-1</sup>) showed the maximum densities of propagules, beyond the accumulation of propagules at the upper limit of salt marshes. Nevertheless, higher biodiversity in the standing vegetation in less stressful habitats was not matched by higher biodiversity in their seed banks, probably due to the combined effects of the temporary nature of the seed bank of most species and the

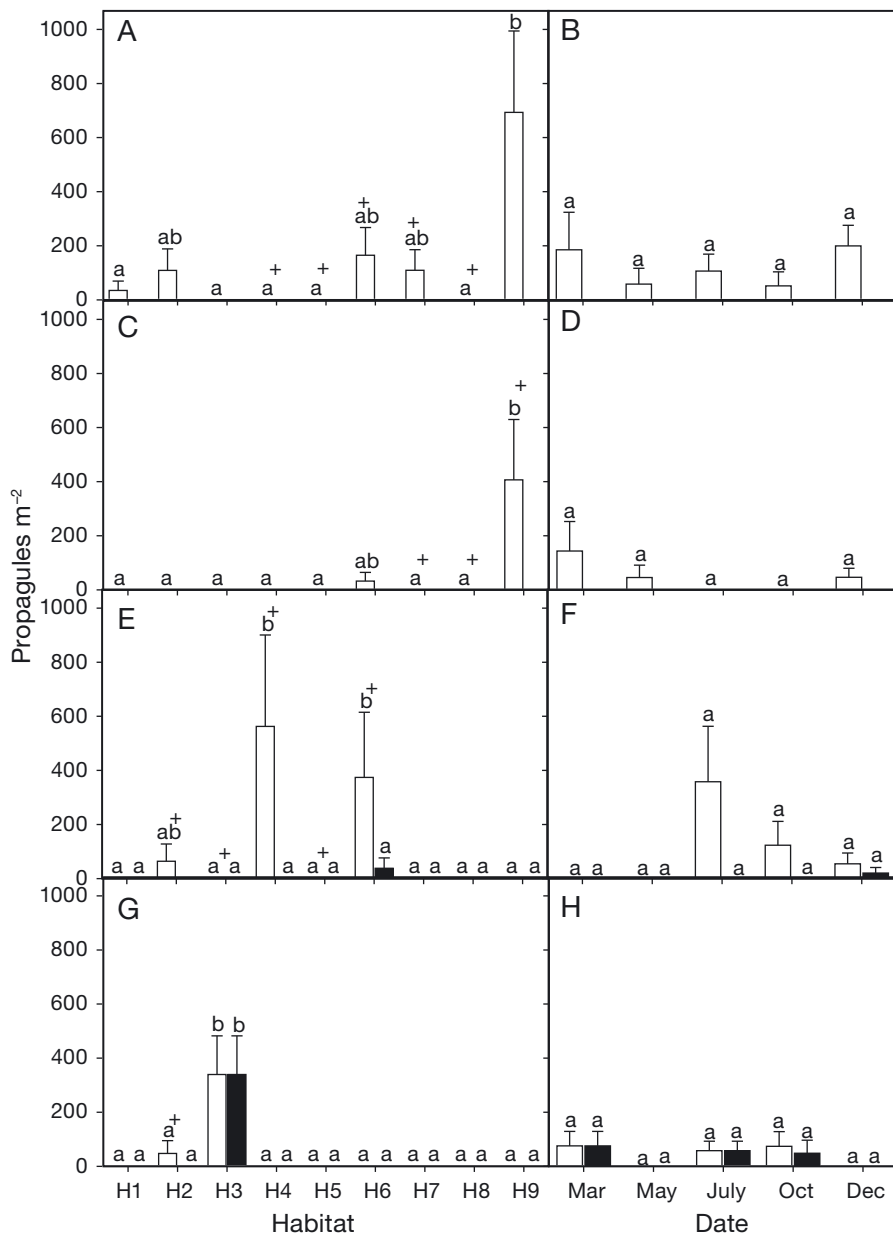


Fig. 3. Density of propagules (white bars: total; black bars: containing seed with embryo) of (A,B) *Limonium ferulaceum* plus *L. diffusum*, (C,D) *Salsola vermiculata*, (E,F) *Halimione portulacoides* and (G,H) *Salicornia ramosissima* in the sediments at 9 salt marsh habitats (H1–H9) along the intertidal gradient in 5 months (March, May, July, October and December) at Ría Formosa (southwest Iberian Peninsula). (+) indicates presence of the species in the standing vegetation at each habitat. Different lowercase letters indicate significant differences between habitats or dates (Mann-Whitney *U*-test,  $p < 0.05$ ). See Fig. 2 for habitat legend. Values are mean  $\pm$  SE ( $n = 45$  samples habitat<sup>-1</sup> H2–H9 and  $n = 60$  in H1;  $n = 84$  samples date<sup>-1</sup>)

dispersal of their propagules.

Our results showed that the halophytes established along the intertidal gradient presented 3 dispersal patterns. (1) Short-distance dispersal was shared by *Sarcocornia* sp., *Cistanche phelypaea*, *Halimione*

*portulacoides* and *Salicornia ramosissima*, with their propagules being more abundant at their own habitats than at further distances. The seed coat of both *Sarcocornia* species and *S. ramosissima* (easily liberated from a membranous pericarp), and the bracteoles covering the fruit of *H. portulacoides*, have irregular exotesta projections, hooked hairs or protuberances (Valdés et al. 1987, Davy et al. 2006) that would help them to anchor to sediments and vegetation. In this sense, Kaminsky et al. (2015) reported short-distance dispersal of *S. perennis* seeds, which limited their colonization, but which would also enable them to disperse to medium distances, colonizing the centres of *Spartina maritima* tussocks during the succession development (Castellanos et al. 1994), followed by short-distance dispersal of the hybrid *S. perennis*  $\times$  *fruticosa* (Figueroa et al. 2003). (2) Medium- to long-distance dispersal was shared by *S. maritima*, *S. vermiculata* and *I. crithmoides*, which seemed to disperse their seeds 100s of m away in the studied marshes and beyond the study area. Only one *S. maritima* propagule was recorded in the sediments, although this cordgrass produced high amounts of spikelets (A. F. Muñoz-Rodríguez pers. obs.) that were able to float away transported by currents and tides (Xiao et al. 2016). This dispersal of *S. maritima* along the estuarine channels would be facilitated by its presence at the lower distribution limit of the marshes (Castillo et al. 2000). In the case of *S. vermiculata*, some pieces of the perianth surrounding the fruit become accrescent, developing wing structures useful for wind dispersal (Toderich et al. 2012). This could explain, together with low fructification in the studied area (A. Polo-Ávila pers. obs.),

the low number of seeds recorded in the studied area since winged seeds may be transported to other marshes. *I. crithmoides* also presents anemocorous propagules adapted to dispersion by wind (cipselas crowned by a pappus of hairs) (Devesa 1987a), which

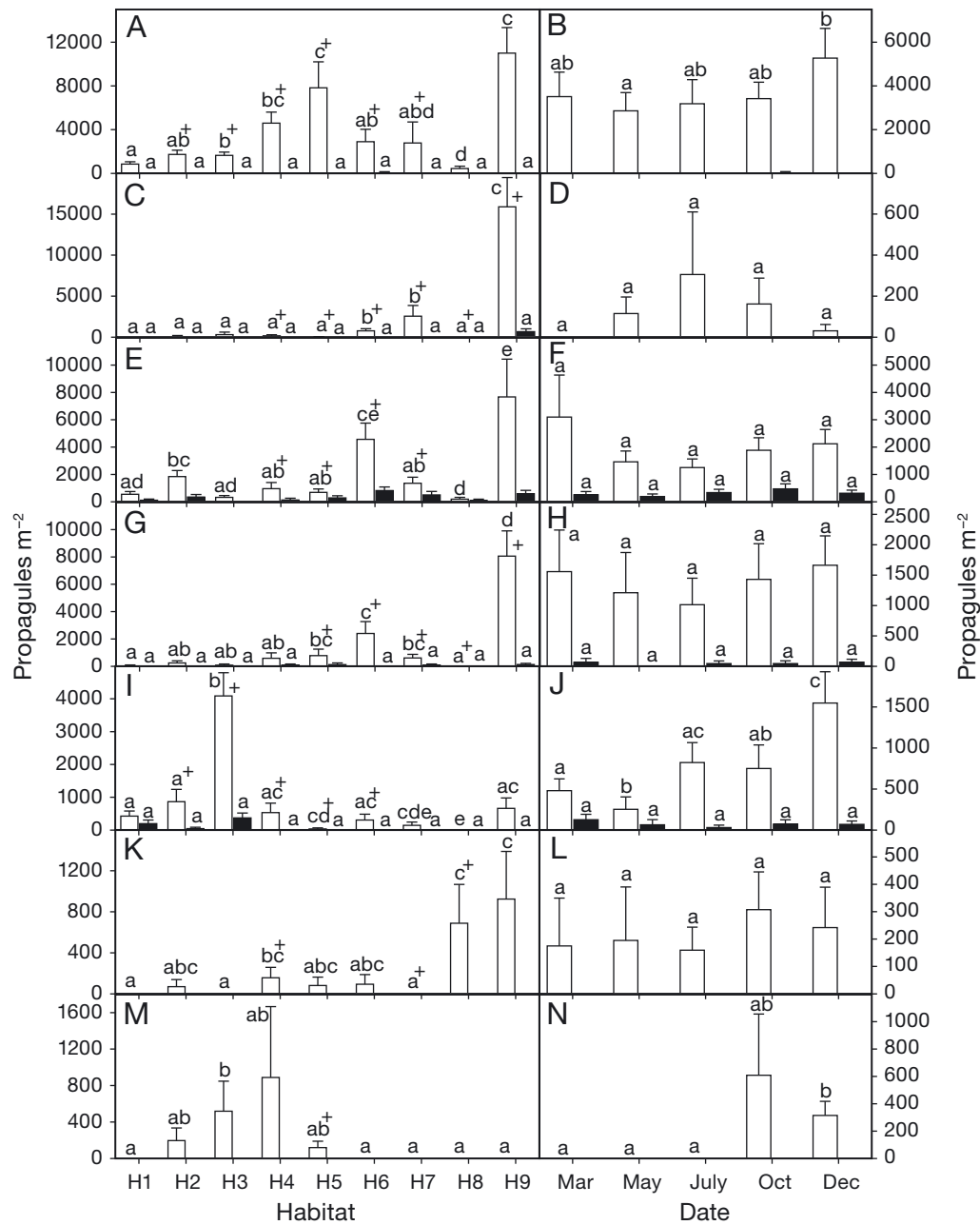


Fig. 4. Density of propagules (white bars: total; black bars: containing seeds with embryo) of (A,B) *Suaeda* sp., (C,D) *Limnias-trum monoptalum*, (E,F) *Arthrocnemum macrostachyum*, (G,H) *Limonium algarvense*, (I,J) *Sarcocornia* sp., (K,L) *Spergularia* sp., and (M,N) *Cistanche phelypaea* in the sediments at 9 salt marsh habitats (H1–H9) along the intertidal gradient in 5 months at Ría Formosa (southwest Iberian Peninsula). (+) indicates the presence of the species in the standing vegetation at each habitat. Different lowercase letters indicate significant differences between habitats or dates (Mann-Whitney *U*-test,  $p < 0.05$ ). See Fig. 2 for habitat legend. Values are mean  $\pm$  SE ( $n = 45$  samples habitat<sup>-1</sup> H2–H9 and  $n = 60$  in H1;  $n = 84$  samples date<sup>-1</sup>)

would explain its low fruit density in the sediments. Diggory & Parker (2011) recorded that a large amount of seed rain was washed out of salt marshes before it entered the seed bank. (3) The behaviour based on a mix of short- and medium- to long-distance dispersal was shared by most of the studied halophytes, as indicated by the accumulation of their

propagules at the upper ecotone of the marshes (between 41 and 597 m away from the mother plants) and at their own habitats.

In total, 12 of the 18 identified propagule (66.6%) showed a clear temporal pattern in their abundance, reflecting seasonality in fruit production, with most of the species dispersing during the autumn. Thus, the

majority of species had transient seed banks, which was reflected in lower species richness and diversity in the seed bank than in the established vegetation at middle and high marshes. Ten taxa (55.5%) showed most of their propagules without any seeds or with damaged embryos, these being propagules left be-

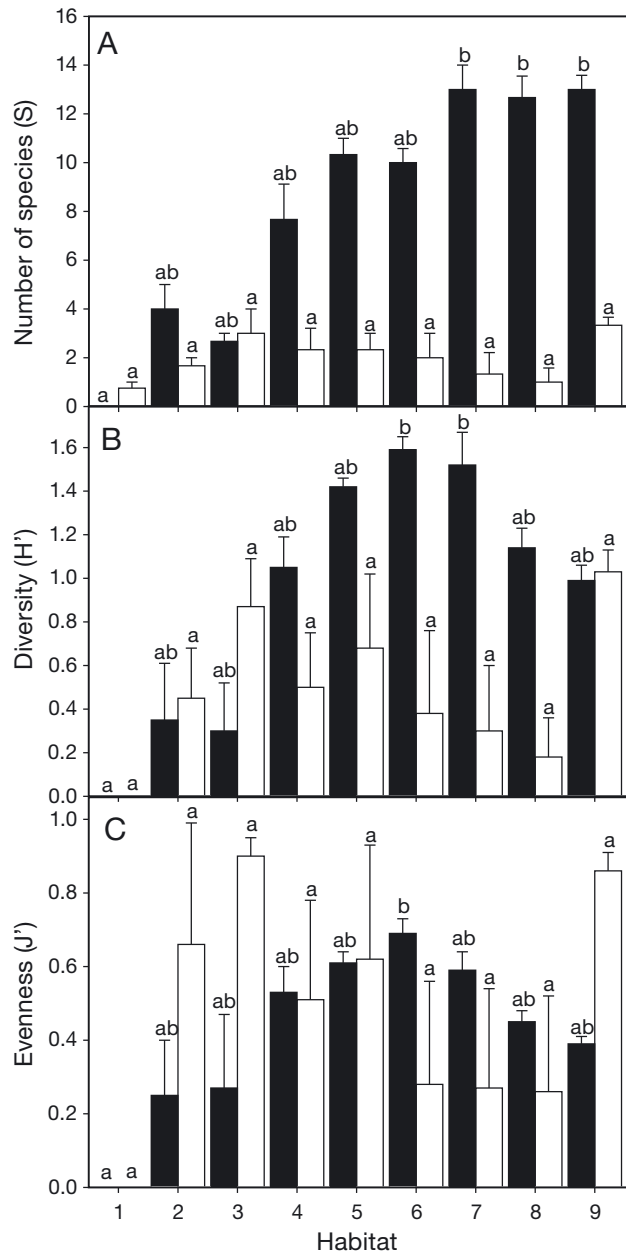


Fig. 5. (A) Species richness, (B) biological diversity and (C) evenness of the halophytic vegetation (black columns) and its seed bank (white columns) at 9 salt marsh habitats (H1–H9) along the intertidal gradient at Ría Formosa (southwest Iberian Peninsula). Different lowercase letters indicate significant differences between habitats (Mann-Whitney  $U$ -test,  $p < 0.05$ ). See Fig. 2 for habitat legend. Values are mean  $\pm$  SE ( $n = 3$ –4 samples habitat $^{-1}$ )

hind after germination. In fact, many seedlings of different halophytes were recorded along the intertidal gradient (A. F. Muñoz-Rodríguez pers. obs.). The vast majority of the seeds of *Suaeda* sp. had no embryo (99.8%), and those seeds with embryo were recorded only in October just after dispersal. The short sampling period and the low density of seeds with embryo seemed to be the result of high percentages of rapid germination when salinity decreased just after dispersal (Muñoz-Rodríguez et al. 2017), as reported in northwest Atlantic salt marshes (Tessier et al. 2000). Damage to the *Suaeda* sp. embryo after dispersal could be due to rapid dehydration as a result of its crustaceous seed coat (Pedrol & Castroviejo 1990). In contrast to our results, Ungar & Woodell (1993) found *S. vera* forming persistent seed banks in British marshes. On the other hand, every sampled spicule of *Limonium ferulaceum* plus *L. diffusum* and *L. narbonense* was empty whereas these halophytes presented, prior to dispersal, ca. 8, 24 and 20% of viable seeds, respectively (J. M. Castillo pers. obs.). This was probably related to the rapid germination of *Limonium* species (Monllor et al. 2017). Equally, every sampled propagule of *S. vermiculata* was empty probably due to rapid and high germination (ca. 90% within 1–6 d) when sediment salinity dropped, with or without perianth (Muñoz-Rodríguez et al. 2017). This salinity decrease happens during their dispersal period of *S. vermiculata* in the autumn (Contreras-Cruzado et al. 2017), when seedlings have been observed in the field (A. F. Muñoz-Rodríguez pers. obs.). This runs counter to Bhatt et al. (2017), who stated that *S. vermiculata* seeds remain dormant with their perianth wing. On the other hand, the *H. portulacoides* propagules stayed in the sediments for no more than a few months (fruits were recorded from July to December), and those that contained seeds with embryo, only for a few weeks (recorded only in December). The rapid decomposition of the *H. portulacoides* propagules just after dispersal would respond to the soft membranous nature of their fruit and seed covers; the fleshy bracteoles covering the fruits promote germination under optimal conditions (Redondo-Gómez et al. 2007, Muñoz-Rodríguez et al. 2012). *H. portulacoides* and *S. vermiculata* fruits covered with bracteoles and calyx, respectively, showed lower and slower germination than when they were devoid of these permanent structures (Muñoz-Rodríguez et al. 2017). The seeds of the holoparasite *C. phelypaea* were also recorded only from October to December just after dispersal. Our results coincide with Erfanzadeh et al. (2010), who found that most of the perennial species were absent from



the persistent seed bank in northwest European salt marshes. The halophytes recorded with transient seed bank showed fast seed germination with little or no endosperm and thin seed coats. These seed traits are characteristic of permanent high-stress habitats such as salt marshes (Parsons 2012).

Six taxa (*Limoniastrum monopetalum*, *Limonium algarvense*, *A. macrostachyum*, *Sarcocornia* sp., *Salicornia ramosissima* and *Spergularia* sp.) established persistent seed banks, showing undamaged seeds all year round. All the propagules of these species were among the most abundant in the sediments. Fruits containing seeds with *L. monopetalum* embryo were found during most of the year, which may relate to their broad flowering period from April to November and their coriaceous seed coat protecting the embryo (Devesa 1987b). *L. algarvense* presented 3.2% of its spicules containing seeds with embryo, when ca. 34% of its fruits showed viable seeds on the mother plants (J. M. Castillo pers. obs.). Thus, although there was a significant loss of seeds from the source plant to the seed bank, this high marsh halophyte was able to retain some coriaceous seeds in the sediments throughout the seasons. *A. macrostachyum* presented seeds covered with a coriaceous testa that needed to be scarified prior to showing high germination levels (ca. 90%) (Muñoz-Rodríguez et al. 2017). This need to scarify in order to germinate would allow *A. macrostachyum* to form a persistent seed bank, as reported previously in Pakistani salt marshes (Gul & Khan 1998, 2001). Although both *Sarcocornia* species disperse their seeds during the autumn (Valdés et al. 1987), seeds with embryo were found all year round at low and middle marshes, where they would be permanently hydrated and in quiescence due to high salinities (20–30 mS cm<sup>-1</sup> following Contreras-Cruzado et al. 2017) that would limit their germination to values below 50% (Muñoz-Rodríguez et al. 2017). Riddin & Adams (2009) also recorded a persistent seed bank for *S. perennis* in South African salt marshes. In the case of the annuals *Salicornia ramosissima*, *Spergularia marina* and *Spergularia heldreichii*, a persistent seed bank would favor their presence in the variable marsh environment (Ungar & Woodell 1996, Márquez-García et al. 2013). In this sense, previous studies reported that *S. ramosissima* and *S. marina* were able to establish persistent seed banks even after favorable conditions (low salinity, and an adequate amount of light in the case of *S. marina*) encouraged the germination of most of their seeds (Wolters & Bakker 2002, Rubio-Casal et al. 2003, Carter & Ungar 2004).

Halophytes established along the intertidal gradient showing contrasted germination syndromes (Muñoz-Rodríguez et al. 2017) and varied abiotic requirements (Contreras-Cruzado et al. 2017) also presented different dispersal behaviors along the intertidal gradient. We detected much lower species richness and diversity in the seed bank than in the aboveground vegetation, showing that the persistent seed bank did not play an important role in sustaining the diversity of the studied salt marshes, as opposed to less stressed and more diverse ecosystems (Valko et al. 2014, Zepeda et al. 2014). In this context, sediment conditions would be key for the distribution of most halophytes with transient seed banks through its effects on seedling establishment, which was reflected in a complex mosaic of abiotic conditions and vegetation patches along the intertidal gradient (Contreras-Cruzado et al. 2017). Climate change and its effects on rainfall, temperature and salinity (through sea level rise) may compromise the persistence of those many halophytes with transient seed banks, impacting on their germination and establishment (Ooi 2012).

We detected that most of the taxa studied were able to disperse their propagules to medium to long distances (to 100s of m away and probably further), which would contribute to the colonization of new, restored and disturbed habitats. Therefore, the resilience or ability of the marshes studied to regenerate after disturbances relied mainly on seed dispersal and *in situ* rapid germination from transient seed banks, and not on germination from the scarce persistent seed bank, as reported for late successional species (Bossuyt & Honnay 2008). Moreover, this resilience may be compromised by limitation in both propagule dispersal and seed bank functioning. In this sense, the colonization of disturbed habitats could be limited by the recorded rapid germination of many species under favorable conditions (Muñoz-Rodríguez et al. 2017), and colonization would be slow for those species showing mainly short-distance dispersal. In addition, key bioengineering species for salt marsh succession development such as *S. maritima* (Castellanos et al. 1994) were absent from the persistent seed bank. Also, the capacity of disturbed halophyte communities to regenerate from the seed bank would depend on the season the disturbance took place since the transient seed bank of most species is only supplemented by fresh seeds a few weeks a year. In view of our results, the conservation of well-preserved salt marsh areas is key for an integral management strategy, since they may act as reference sites and sources of propagules for passive and

active ecological restoration projects. In this context, the ecotone between salt marshes and adjacent coastal dunes use to be specially impacted by human infrastructures such as roads. This transitional habitat should be preserved since it appears as a suitable area for collecting seeds of halophytes when they cannot be found directly on standing vegetation.

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