Universidad de Huelva

Departamento de Biología Ambiental y Salud Pública

Aspectos reproductivos de la flora de dunas y marismas de las costas de Huelva (SO España)

Reproductive aspects of the flora of dunes and marshes on the coast of Huelva (SW Spain)

Memoria para optar al grado de doctor
presentada por:

Alejandro Polo Ávila

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1. Resumen tesis doctoral

1.1 Introducción

1.1.1 Los hábitats estudiados

El siguiente estudio de doctorado se centra en ampliar el conocimiento de especies que habitan en marismas mareales y su ecotono formado por las dunas colindantes. Tal es así que el ecotono marisma-duna se encuentra recogido en el segundo artículo perteneciente a esta tesis, mediante la recolección de semillas de psamófilos que se acumulaban en este hábitat sumidero. Sin embargo, debido a su interés, los artículos restantes se han centrado en especies puramente marismeñas ocupando, como es lógico, mayor importancia en esta introducción y en el resto de la tesis.

Las marismas saladas son ecosistemas que se encuentran distribuidos por todo el planeta, generalmente en latitudes medias y altas, ocupando un total del 0,3% de la superficie global del planeta y representando el 5% de los humedales del mundo (Zedler et al., 2008; Mcowen et al., 2017). Se distribuyen desde la zona subártica hasta la zona tropical, aunque tienden a desarrollarse más en las zonas templadas del planeta, conviviendo en los lugares tropicales con los manglares, donde las marismas quedan relegadas a las zonas adyacentes más altas (Broome et al., 1988; Chmura, 2009). Las marismas mareales se situan en zonas de costa protegidas, donde la energía física del oleaje es lo suficientemente baja como para permitir el establecimiento de una vegetación tolerante a la sal en la zona intermareal (Pethick, 1984; Reed, 1990). Son ecosistemas cambiantes, cuyos principales motores de cambio son la amplitud de las mareas y el aporte constante de sedimentos de diferentes fuentes, principalmente los arrastrados por las mareas y los depositados por los ríos en su desembocadura. Ambos factores de cambio son los que favorecen el asentamiento de comunidades vegetales que a su vez, aumentan el ritmo de deposición de sedimentos y la evolución dinámica del ecosistema hacia un medio puramente terrestre (Friedrichs and Perry, 2001). Debido principalmente a este aporte continuado de sedimentos/nutrientes, las marismas mareales son uno de los ecosistemas más productivos del mundo, derivando de ello una gran variedad de servicios ecosistémicos (Pennings and Bertness, 2001). Estos servicios son definidos como los beneficios que obtenemos los seres humanos de los sistemas ecológicos y que se generan a partir de los procesos y funciones de los ecosistemas, como la producción primaria y secundaria, la descomposición y la transformación de nutrientes (Brown et al, 2006). Estos beneficios son múltiples y muy diversos, como actuar como barreras naturales en las zonas de costa previniendo la erosión de las mismas limitando a su vez las inundaciones de ciudades y pueblos costeros (King and Lester 1995, Moeller et al. 1996); también actúan como sumideros de nitrógeno, limitando su entrada a los acuíferos (Valiela and Teal, 1979), además de proporcionar un importante servicio para el recreo y el esparcimiento. Pero no sólo los humanos obtenemos rentabilidad de estos ecosistemas, las marismas proporcionan hábitat, refugio y alimento a multitud de especies de peces, crustáceos y aves entre las que encontramos las migradoras (Boesch and Turner, 1984; Brown et al, 2006).

Aun con todos los beneficios y aprovechamientos que obtenemos de las marismas, estos ricos ecosistemas no se hayan exentos de amenazas derivadas de la actividad del hombre como la destrucción originada por la ocupación y pérdida de tierras para otros usos como la agricultura o la ampliación de las urbes; también son susceptibles a la eutrofización, debido a la aplicación de fertilizantes en los campos a lo largo de las cuencas hidrográficas de los ríos que acaban en los estuarios como contaminantes; otra amenaza es derivada de la subida del nivel del mar debido al cambio climático; y sufren el asentamiento de especies invasoras, como es el caso de *Spartina densiflora* en las marismas onubenses (Jorgensen, 2009), entre otras amenazas.

Las marismas mareales presentan una zonación espacial que se manifiesta en el asentamiento de distintas y diversas especies en función de su tolerancia a los factores abióticos, así como a la interacción entre dichas especies (Chapman, 1974; Vince and Snow, 1984). Dicha zonación viene determinada principalmente por la acción de las mareas, que actúa de forma diferencial en las distintas elevaciones de las marismas con respecto al nivel del mar (Adam, 1990; Callaway et al., 1990; Davis et al., 1996; Apaydin et al., 2009). La fluctuación de las mareas con el ciclo lunar y las estaciones, junto con las distintas elevaciones del terreno que encontramos en la marisma, determinan marcados gradientes abióticos entre las cotas topográficas inferiores y superiores de las marismas, observándose este efecto particularmente en el sedimento (Adam, 1990), concretándose en cambios en su textura y estructura, el contenido en agua, el pH, la oxigenación del suelo y la salinidad, (Cantero et al., 1998; Cott et al,. 2013; Contreras-Cruzado et al., 2017), siendo estos los factores que afectan de una manera mas marcada al establecimiento de la vegetación en la marisma (Cantero et al., 1998; Onaindia and Amezaga, 1999). Según Contreras-Cruzado et al. (2017), encontramos los mayores contenidos de agua y materia orgánica del suelo en los suelos desnudos de marisma baja y en las comunidades de marisma media. El pH y la conductividad del suelo, son dos factores con una alta temporalidad debido a las condiciones del clima mediterráneo, con inviernos frescos y lluviosos y veranos cálidos y secos que provocan cambios debidos a la evaporación de gran parte del agua contenida en los sedimentos en los meses más caluroros, y el lavado de sales por escorrentía superficial en los meses más lluviosos. Esto provoca que se concentren los mayores cambios estacionales de salinidad del suelo en las comunidades de las zonas media y media-alta de marisma, coincidiendo con las zonas de mayor contenido en agua y que sufren más debido a la evaporación de la misma en época estival. A grandes rasgos en cuanto a los niveles de pH, Contreras-Cruzado et al., (2017) encontraron niveles de pH neutro en las zonas media y baja y pH alcalinos en las zonas altas del ecosistema marismeño.

La concentración de sales en el suelo es uno de los factores abióticos más limitantes para el asentamiento de una especie vegetal, por lo que es un factor fundamental en la organización de la vegetación de las marismas, actuando mediante una red de gradientes espacio-temporales, que dependen de la elevación del sustrato con respecto al marco intermareal (Egan and Ungar, 2000). Estos es debido a que la salinidad del medio condiciona en muchas especies las primeras etapas de su desarrollo, como es el caso de la inhibición de la germinación de las semillas, y también el posterior establecimiento de las plántulas, que se ha descrito como una fase crucial para la colonización de algunas plantas dentro del ambiente salino estresante de la marisma (Rubio-Casal et al., 2001). A grandes rasgos, la alta concentración de sal interrumpe la homeostasis en el potencial hídrico de la planta y la distribución de iones a nivel celular, lo que lleva a la degradación de las moléculas biológicas, la detención del crecimiento y la muerte celular (Munns and Tester, 2008).

En este ambiente estresante son frecuentes las llamadas especies halófitas (Ungar, 1987), consideradas como aquellas especies de plantas que son capaces de permanecer vivas y completar su ciclo vital con concentraciones de sal de agua de mar (Rengasamy et al., 2003; Flowers and Colmer, 2008; Fita et al., 2015). Para sobrevivir y desarrollarse en un ambiente con concentraciones de sal que sería letales para el 99% del resto de plantas del planeta (Trotta et al., 2012), las plantas halófitas emplean estrategias para su supervivencia entre las cuales caben citar la captación y retención de NaCl en las vacuolas, provocado por un aumento del eflujo de sódio en la membrana plasmática en respuesta a altas salinidades, reteniendo los iones monovalentes tóxicos (Na^+ y Cl^-) en las vacuolas y manteniendo sus concentraciones en el citoplasma dentro de niveles tolerables (Zhu, 2000; Subudhi and Baisakh, 2011); Otro mecanismo son las llamadas glándulas de sal, que son unas estructuras que se concentran en las partes aéreas de la planta, sobre todo en las hojas, y son capaces de expulsar agua con altas concentraciones de sal disueltas, la cual queda adherida a la hoja formando una especie de costra blanquecina que será lavada por las lluvias o arrastradas por el viento (Waisel, 1972; Lipscshitz et al., 1974); Además, las semillas de las especies halofitas han desarrollado mecanismos de latencia para retrasar la germinación cuando las concentraciones de sal en el medio son desfavorables para el desarrollo de la plántula (Khan and Gul, 2006).

1.1.2 Las especies estudiadas

Como se citó anteriormente, la presente memoria de tesis se centra en el estudio de especies pertenecientes a las comunidades de marismas mareales, comunidad estudiada al completo en el trabajo de dispersión de propágulos, llegándose a indentificar y estudiar dieciocho tipos de propágulos. Para el estudio de los efectos de la salinidad en cuanto a la germinación de semillas, se eligieron especies pertenecientes a la familia Chenopodiaceae, por su importancia dentro de las comunidades de marisma. Por último, decidimos realizar dos estudios centrándonos en la especie anual *Salicornia ramosissima*, especie que está alcanzando un alto interés comercial y gastronómico, y de la que estudiamos la dinámica de su banco de semillas, así como la dinámica poblacional en general, y que describiremos a continuación.

El género *Salicornia* está formado por plantas anuales con tallos articulados y hojas opuestas soldadas entre si, formando un artejo craso. Presentan inflorescencias espiciformes con dos cimas triflorares opuestas en cada segmento, con la flor central separada de las dos laterales y semillas verticales oblongas u ovoideas, con pelos uncinados. Dentro de este género se encuentra la especie *Salicornia ramosissima*, una especie que se distingue por presentar artejos fértiles con los lados convexos (forma de tonel), presentando su anchura máxima normalmente en la mitad superior y con flor central de cada cima más grande que las laterales. Se encuentra ampliamente distribuida por el Oeste de Europa y el Noroeste de África, estando presente prácticamente en toda la Península Ibérica y Baleares (Valdés, 1987). Es una especie colonizadora de las marismas saladas donde aparece en una amplia variedad de hábitats de marisma, con preferencia por los bordes de los charcones hipersalinos que se forman por acumulación y posterior evaporación de agua salada en las marismas, siendo sus semillas capaces de germinar en altos niveles de salinidad (Chapman, 1974; Davy et al., 2001; Rubio-Casal et al., 2003; Muñoz-Rodríguez et al., 2017). La importancia socio-económica de esta especie radica en que tiene diferentes usos tradicionales como alimento, empleando sus tallos y hojas en diversas elaboraciones como ensaladas o patés (Patel, 2016; Loconsole et al., 2019), también se emplea como recurso farmacológicos (Barreira et al., 2017; Lefèvre et Rivière, 2020; Lima et al., 2020; Cárdenas-Pérez et al., 2021) entre otros. Además, tiene otros usos no tradicionales entre los que destacar su empleo como biofiltro para reciclar el agua y los nutrientes contenidos en los efluentes de la acuicultura marina (Cárdenas-Pérez et al., 2021), teniendo una alta tolerancia al crecimiento en suelos contaminados por metales (Márquez-García et al., 2013) habiendo sido propuesta para fitorremediación, debido a su capacidad de acumular Cd en sus raíces (Pedro et al., 2013).

1.2 Objetivos

Los objetivos que se pretenden alcanzar con la realización de esta tesis doctoral son los siguientes:

- Caracterizar las estrategias de dispersión de propágulos de las distintas especies halófitas que colonizan las marismas mareales del suroeste de España.
- Estudiar la capacidad germinativa de las semillas de las especies halófitas en distintas concentraciones salinas.
- Determinar la existencia o no de bancos de semillas permanantes en el suelo de las especies halófitas.
- Estudiar el banco de semillas de la especie anual *Salicornia ramosissima*.
- Determinar los factores que afectan a la dinámica poblacional de la especie anual *S. ramosissima*.

1.3 Material y métodos

El material vegetal necesario para la elaboración de todos los trabajos contenidos en esta tesis ha sido recolectado en marismas mareales situadas en el Golfo de Cádiz (suroeste de la Península Ibérica):

- En los trabajos en los que se necesitaba una diferenciación de las comunidades a lo largo del gradiente mareal, los estudios se llevaron a cabo en marismas de la Isla de Tavira (37º 05' N 7º 40' O), situadas en el Parque Natural da Ria Formosa (Algarve, Portugal) (Muñoz-Rodríguez et al., 2017; Polo-Ávila et al., 2019), debido a su escasa pendiente, lo que facilitaba la separación de las comunidades Contreras-Cruzado et al. (2017).
- Por su parte, los estudios del banco de semillas y de la dinámica poblacional de *S. ramosissima* (Polo et al., 2021; Polo-Ávila et al., 2022), se realizaron en las marismas mareales del Paraje Natural Marismas del Odiel (37º 12' 22.3'' N, 6º 58' 01,5'' W) (Huelva, España).

El protocolo de recogida y tratamiento de las muestras fue el mismo para los cuatro estudios, y se expone quedando resumido a continuación:

- Las temperaturas medias, máximas y mínimas del aire y la precipitación diaria durante el período de estudio fueron facilitadas por la estación meteorológica ubicada en la avenida Francisco Montenegro 37º 16' 00'' N 06º 57' 00'' W a 15 m sobre el nivel del mar (Huelva).
- Los suelos para los trabajos de determinación de banco de semillas fueron recolectados empleando un anillo de acero inoxidable de 50 mm de diámetro y altura, y almacenados en bolsas herméticas de polietileno a -20 ºC hasta su estudio. Para la determinación del banco de semillas se dividieron las muestras iniciales en 6 sub-muestras de 5 g cada una, eliminando de ellas mediante filtración y lavado las partículas de suelo y grava no deseadas. El material restante fue examinado bajo lupa consiguiendo así discriminar, clasificar y cuantificar las semillas contenidas en las muestras.
- Para el estudio de los factores edáficos tales como pH, conductividad, materia orgánica y contenido en agua, los suelos fueron recogidos empleando un anillo de acero inoxidable de 50 mm de altura y diámetro, siendo almacenados a – 20 ºC en bolsas de polietileno herméticas hasta su estudio. La conductividad del suelo fue empleada como medida de la salinidad del mismo (Richards, 1974) y para determinarla se empleó un conductivímetro (Crison Instruments 5064, Hach Lange, Barcelona, España) sobre una mezcla homogénea 1:1 en volumen de 10 ml de suelo y agua destilada; El pH fue medido en la misma mezcla homogénea, empleando en este caso un pHmetro (Crison 25, Hach Lange, Barcelona, España) (Nieva et al., 2001). El contenido de agua de los suelos se determinó mediante gravimetría empleando muestras de 30 g (Contreras-Cruzado et al., 2017). En cuanto al contenido de materia orgánica en los suelos fue determinada mediante el método "loss-on-ignition", siendo calculado el contenido en materia orgánica en suelos, previamente secos, mediante la proporción de perdida de peso comparando los suelos antes y después de la ignición (Gavlak et al., 2005).
- La recolección de semillas y frutos para la realización de ensayos de germinación se realizó para cada especie en el momento de madurez idóneo, que es diferente para las distintas especies estudiadas (Fernández-Illescas et al., 2010). Una vez recolectados, eran limpiados y almacenados en bolsas de papel a 25 ºC y condiciones de oscuridad, por un periodo no superior a 2 meses.
- Para los estudios de germinación las semillas fueron esterilizadas mediante inmersión en solución de hipoclorito sódico al 5% (v/v) durante diez minutos (Muñoz-Rodríguez et al., 2012), transcurrido los cuales fueron lavadas con abundante agua

destilada y dispuestas en placas de Petri de 9 cm de diámetro, junto con 3 hojas de papel de filtro autoclavado y empapadas con 5 ml de las distintas soluciones de estudio, y selladas a su vez con Parafilm$^{\mathrm{TM}}$. Para cada uno de los taxones contó con tres placas de Petri por tratamiento, con 25 semillas en cada placa. Dichas placas se mantuvieron bajo un fotoperiodo de 12 horas de luz/12 horas de oscuridad, con un ciclo de temperaturas de 24/20 ºC. Se registró la germinación en las placas durante 30 días, con una temporalidad de 2 días entre registro. Para estudiar la recuperación de las semillas tras su exposición en distintas concentraciones salinas, las semillas no germinadas en las concentraciones iniciales fueron lavadas con agua destilada, nuevamente esterilizadas y sembradas en agua destilada de nuevo por un periodo de 30 días.

- Para los estudios de dinámica poblacional de *S. ramosissima* se eligieron cuatro poblaciones y se marcaron tres zonas en cada una de ellas, colocándose en cada zona 5 anillos de plástico de 4,5 cm de diámetro, anclados al sustrato con 2 estacas cada uno. En 11 fechas desde la germinación incial hasta le fructificación, se registró el número de plantas de *S. ramosissima* dentro de cada anillo. En la última de las fechas se contaron las plantas que habían florecido y se midió la altura de todas las plantas desde la base hasta el ápice de la planta. Se calculó la densidad de plantas por unidad de superficie para cada anillo de muestreo en cada fecha de muestreo y la densidad máxima registrada en cada anillo; se calculó la proporción final de plantas sobrevivientes en la última fecha de muestreo en relación con la densidad máxima de plantas alcanzada para cada anillo; y se calculó la proporción de plantas en flor en relación con la densidad total de plantas en la última fecha de muestreo y con la densidad máxima de plantas alcanzada. Además, se calculó la variación diaria de la densidad de plantas en relación con la densidad máxima para cada anillo y su relación con la densidad máxima alcanzada y con las variaciones de distintos parámetros ambientales (precipitación, temperaturas, pH y conductividad).
- Los análisis estadísticos fueron realizados mediante el programa estadístico STATISTICA 8.0 (Statsoft), tomando como nivel de significancia $p < 0,05$. La normalidad de la serie de datos se probó mediante la prueba de Shapiro-Wilk y la homogeneidad de la varianza mediante la prueba de Levene. Como los datos no presentaban homogeniedad ni normalidad, se probaron tres transformaciones utilizando las siguientes funciones $1 / (x + 1)$, $\log(x + 1)$, $\arcsin(x)$ y √–x, y como los datos continuaban sin seguir una distribución normal o mostrar homogeneidad en sus varianzas, se emplearon para su comparación las pruebas no paramétricas Kruskal-Wallis H-test y Mann-Whitney U-test como pruebas post hoc.

1.4 Discusión de resultados

El conjunto de los cuatro estudios presentados en esta tesis doctoral abarca veintidós taxones de especies halófitas presentes en las marismas del Golfo de Cádiz al suroeste de la Península Ibérica, que se enumeran a continuación: *Arthrocnemum macrostachyum* (Moric.) K. Kock., *Atriplex halimus* L., *Cistanche pelypaea* (L.) Cout., *Halimione portulacoides* Aelen., *Inula crithmoides* L., *Spergularia heldreichi* Foucaud, *Spergularia media* (L.) C. Presl., *Salicornia ramosissima* J.Wood., *Salsola vermiculata* L., *Sarcocornia fruticosa* (L.) A.J.Scott, *Sarcocornia perennis* (Mill.) A.J.Scott, *Suaeda albescens* Lázaro Ibiza, *Suaeda vera* Forssk., *Frankenia laevis* L., *Limoniastrum monopetalum* (L.) Boss., *Limonium algarvense* Erben., *Puccinelia convoluta* (Hornem.) Fourr., *Spartina maritima* (Courtis) Fernald., *Poligonum equisetiforme* Sibth and Sm., *Limonium ferulaceum (L.) Chaz., Limonium diffusum* (Pourr.) Kuntze y *Limonium narbonense* Mill.

Al estudiar el rango de dispersión de propágulos para cada especies, encontramos que el ecotono entre el ecosistema dunar y marismeño actuaba como sumidero para la mayoría de los propagulos recolectados, siendo esta zona colonizada únicamente por tres especies (*Salsola vermiculata, Frankenia Laevis y Limonium algarvense*) (Contreras-Cruzado et al., 2017), teniendo en contrapunto, como zona de menor carga de propágulos, los sedimentos arenosos adyacentes donde encontramos una comunidad casi monoespecífica de *S. vermiculata*, junto con los fondos desnudos de la marisma baja, lo que va en concordancia con Huiskes et al. (1995) y Wolters and Bakker (2002). Esto es así debido a la acción de sumidero que realiza el ecotono influenciado por la capacidad de dispersión de la mayoría de los propágulos a través del viento o mediante las mareas. Si no tenemos en cuenta la acción sumidero del ecotono, encontramos que siete de los nueve hábitats estudiados tenían su propia composición única de propágulos, observando que las concentraciones de propágulos de cada especie solían encontrarse cerca de las plantas madre como reflejan los análisis de correlación entre cobertura de especies y densidad de semillas, dando como resultado una composición heterogénea del banco de semillas a lo largo del gradiente intermareal (Rand, 2000; Noe and Zedler, 2001).

Analizando los taxones y su comportamiento, encontramos tres patrones de dispersión claros: (1) Definido por una dispersión a corta distancia tomando como punto de partida las plantas madre, presente en *Sarcocornia* sp., *Cistanche phelypaea, Halimione portulacoides* y *Salicornia ramosissima*. Esto es debido a la propia estructura externa que presentan las semillas, que ayudan a estas a anclarse al sedimento y a la vegetación adyacente, permaneciendo cerca de la fuente, concretamente se trata de la presencia de pelos en forma de gancho en la cubierta seminal de *Sarcocornia* y de *S. ramosissma*, y presencia de pelos en proyecciones exoesas irregulares, o protuberancias en las bracteólas que recubren el fruto de *H. portulacoides* (Valdés, 1987; Davy et al., 2006). (2) La dispersión de media a larga distancia se registró en *S. maritima*, *S. vermiculata* e *I. crithmoides*, que dispersan sus semillas a cientos de metros de distancia de las plantas madre e incluso fuera de la zona de estudio. Un ejemplo de la alta capacidad de dispersión lo tenemos en *S. maritima*, de la que se registró sólo un propágulo en los sedimentos, aunque las plantas de dicha especie produjeron grandes cantidades de ellos, esto es debido a la capacidad de flotar y ser transportadas por corrientes y mareas (Xiao et al., 2016). En cuanto a *S. vermiculata* e *I. crithmoides*, ambos propágulos presentan estructuras que facilitarían su dispersión por el viento (Toderich et al., 2012; Devesa, 1987a). (3) Por último encontramos un comportamiento de dispersión mezcla de los dos anteriores, empleado por la mayoría de las halófitas estudiadas como refleja la acumulación de propágulos en el hábitat nueve (ecotono).

En total, 12 de los 18 taxones estudiados para el trabajo de dispersión mostraron un claro patrón temporal en su abundancia, reflejando la estacionalidad en la producción de frutos, unido con que la mayoría de las especies formaron bancos de semillas transitorios, debido a la alta y rápida germinación cuando las condiciones son adecuadas y a la predación. Esta alta y rápida germinación de la mayoría de las especies estudiadas provocó que todas las espículas muestreadas de *Limonium ferulaceum,* *L. diffusum* y *L. narbonense* estuvieran vacías, presentando antes de su dispersión hasta el 20% de semillas viables (Monllor et al., 2017). Del mismo modo todos los propágulos muestreados de *Salsola vermiculata* estaban vacíos, debido a su rápida y alta germinación (aprox 90% de 1-6 días) (Muñoz-Rodriguez et al., 2017), fenómeno que tiene lugar en otoño, momento de su dispersión (Contreras-Cruzado et al., 2017). Estos resultados van en contra de los obtenidos por Bhatt et al. (2017), quienes afirman que las semillas de *S. vermiculata* permanecían latentes en el banco de semillas junto con la estructura del periantio. *H. portulacoides* presentó propágulos en los sedimentos durante sólo unos meses (julio-diciembre), presentando sólo propágulos con embrión en diciembre. Esto respondería a la rápida descomposición de los propágulos tras su dispersión, debido a la naturaleza membranosa y poco duradera de su cubierta, favoreciendo en todo caso la alta tasa y velocidad de germinación cuando la semilla se encuentra desprovista de la bractéola membranosa que la envuelve (Muñoz-Rodríguez et al., 2017).

En cuanto a las especies que presentan bancos de semillas persistentes, encontramos seis taxones (*L. monopetalum, L. algarvense, A. macrtostachyum, Sarcocornia sp., S. ramosissima* y *Spergularia sp.*) que mostraron semillas intactas durante todo el año, siendo además los taxones cuyos propágulos estaban entre los más abundantes en los sedimentos. *L. monopetalum* presentó semillas durante gran parte del año debido principalmente a su amplio periodo de floración, de abril a noviembre, y a su cubierta seminal coriácea que protege al embrión (Devesa, 1987b). *L. algarvense* presentó semillas viables con cubierta coriácea a lo largo del todo el periodo de estudio. La semilla de *A. macrostachyum* presenta una cubierta coriácea que requiere ser escarificada antes de alcanzar niveles de germinación elevados (por encima del 90%) (Muñoz-Rodríguez et al., 2017), es por ello que es requerimiento indispensable la formación de bancos de semillas persistentes (Gul and Khan, 1998, 2001), que garanticen un tiempo de permanencia en el suelo suficiente para que la cubierta sea desgastada por rozamiento. En cuanto a las semillas de *Sarcocornia sp*. aunque dispersan sus semillas en otoño (Valdés et al., 1987), se encontraron semillas viables durante todo el año en marismas baja y media, donde permanecen hidratadas y en letargo debido a las altas salinidades (Contreras-Cruzado et al., 2017), limitando su germinación por debajo del 50% (Muñoz-Rodríguez et al., 2017). En el caso de especies anuales como *Salicornia ramosissima, Spergularia marina* y *S. heldreichii*, la existencia de un banco de semillas persistente favorecería su presencia el ciclo siguiente en un ambiente variable (Ungar and Woodell, 1996; Márquez-García et al., 2013).

En un estudio dirigido específicamente a analizar el banco de semillas de *S. ramosissima*, encontramos que la mayor parte de la producción anual de semillas (> 79%) se exportaba fuera de las poblaciones, acumulándose sólo entre el 14 y el 20% en los bancos de semillas aéreo y del suelo, y que sólo se mantenía un banco de semillas permanente en dos de las cuatro poblaciones de estudio, quedando estas semillas en latencia debido a las condiciones de alta salinidad, sin afectar a la viabilidad de las mismas (Rubio-Casal et al., 2003; Muñoz-Rodriguez et al., 2017).

Dicho banco de semillas podía ser mermado en distintos momentos del año, en respuesta a la alta y rápida germinación de semillas en condiciones favorables (Parson, 2012), y a la depredación de semillas, observada en diferentes especies de *Salicornia* (Davy et al., 2001; Polo-Ávila et al., 2022). Esta estrategia de germinación temporal a nivel de población probablemente se basó en una ruptura escalonada de la latencia fisiológica inducida por altas salinidades (Rubio-Casal et al., 2003; Muñoz-Rodríguez et al., 2017), además de ser una estrategia común a las especies halófitas aprovechando las ventanas de germinación especialmente en hábitats altamente estresantes como las salinas (Gul and Weber, 2001; Noe et al., 2001; Xie et al., 2019). Por lo tanto, este patrón temporal de germinación puede permitir que algunas plantas de *S. ramosissima* eviten eventos catastróficos, como la herbivoría o inundaciones violentas de ríos, que podrían afectar sus poblaciones durante la temporada de crecimiento.

Pero *S. ramosissima* no sólo forma bancos de semillas en suelo, sino que también tiene la capacidad de formar bancos de semillas aéreos, los cuales ayudan a dosificar la dispersión en el tiempo de las semillas, a la vez que las protegen de la predación y las condiciones desfavorables que se dan en el sustrato (Santini and Mantorell, 2013; El-Keblawy and Bhatt, 2015).

Ambos bancos de semillas iniciales mostraron signos de agotamiento durante el ciclo en curso (Polo et al., 2021), acumulando menos del 1% de la producción anual de semillas (de 19 a 15,3 semillas m^{-2}) para el siguiente periodo de fructificación, con lo que, la producción de semillas es clave para la preservación de las poblaciones de *S. ramosissima* que no establecen bancos de semilla en suelo. En este sentido registramos una alta producción anual de semillas (> 48.000 semillas m^{-2}), dependiendo esta producción principalmente de la densidad de plantas más que de la producción de semillas producidas por cada planta individual, la cual era similar para cada población. De hecho, la mayor producción de semillas por planta (604 semillas planta $^{-1}$) se obtuvo en la población con mayor densidad de plantas (3.610 plantas m^{-2}), dando como resultado 2.179.383 ± 614.577 semillas m^{-2}. La dinámica de los bancos de semilla cambió notablemente entre las poblaciones de *S. ramosissima*, siendo el banco de semillas aéreo inicial mayor que el banco de semillas de suelo inicial en tres de las cuatro poblaciones, acumulando más de 2.800

semillas m^{-2} en cada población. Entre el 71,8% y el 99,8% del banco de semillas aéreo inicial se dispersó o fue depredado durante el primer año, mientras que el banco de semillas del suelo como vimos anteriormente, se agotó por completo en dos de las poblaciones. El banco de semillas aérero remanente tendió a aumentar con la densidad de las plantas, almacenando entre 19 y 15.302 semillas m^{-2} en diferentes poblaciones. Por lo que, aunque anteriormente se describió que la estrategia de dispersión de *S. ramosissima* es a corta distancia, en este estudio encontramos que entre el 19,5 y el 85,9% de la producción anual de semillas fue depredada o exportada fuera de las poblaciones. Al haber encontrado menos del 0,9% de semillas en las zonas adyacentes a la población y dada su baja flotabilidad (Huiskes et al, 1995) y las estructuras de anclaje que posee en su superficie como citamos anteriormente, se sugiere que la depredación es más importante que la exportación de semillas fuera de las poblaciones de estudio.

También debemos tener en cuenta para la mayor comprensión y mejor manejo de esta especie su dinámica poblacional, compleja y dependiente de la posición fisiográfica en salinas bajo clima mediterráneo, en relación con las diferencias en la densidad de plantas. En áreas elevadas con buen drenaje, las poblaciones de *S. ramosissima* presentaron densidades máximas de plantas entre 628 y 6.288 plantas m^{-2} que se mantuvieron casi constantes hasta la fructificación. Estas densidades máximas mantuvieron un equilibrio dinámico entre la mortalidad y el establecimiento de nuevas plántulas, con tasas de supervivencia de plantas entre 46-64%. Este patrón de la curva de supervivencia, que podría asimilarse al Tipo I de Deevey (Deevey, 1947), revela que la mortalidad de las plantas individuales se concentró al final de su vida. En contraste, las poblaciones que colonizaban zonas deprimidas, frecuentemente anegadas, presentaron densidades de banco de semillas del suelo y densidades de plantas cinco veces superiores (entre 1.257 y 51.558 plantas m^{-2}) que las poblaciones en zonas elevadas, aunque perdieron c. 60% de sus densidades máximas de plantas desde finales de primavera y durante todo el verano, coincidiendo con el periodo cálido y seco del clima mediterráneo en el que se producen altos valores de evapotranspiración, elevando la concentración de sales en el suelo (Chapman, 1974; Álvarez-Rogel et al., 2000), a la vez que se detecta una alta mortalidad de plantas de marisma (Kaminsky et al., 2015).

En cuanto a la respuesta de las semillas de las plantas halófitas de la familia Chenopodiaceae frente a la salinidad, de manera generalizada, un aumento de la salinidad inhibe la germinación y fuerza la inactividad de las semillas (Pujol et al., 2001), siendo esto un mecanismo de defensa que permite a estas plantas evitar condiciones estresantes que pongan en peligro el desarrollo de las plántulas (Song et al., 2005). La germinación tendió a acelerarse (menor T50) en agua dulce tras una exposición a altas concentraciones de sal, lo que se observó en todas las especies estudiadas salvo en *A. macrostachyum* y *S. vera* que dispersan sus semillas en primavera, mientras que el resto las dispersan en otoño-invierno, por lo que verían reducida drásticamente la exposición a altas concentraciones de sal con el lavado debido a las lluvias, favoreciendo que ocupen el espacio disponible antes que otras especies (Pujol et al., 2001; Navarro and Guitián, 2003; Redondo et al., 2004). Pero más allá de las respuestas comunes, las principales plantas halófitas de la familia Chenopodiaceae que encontramos en el Golfo de Cádiz mostraron cuatro tipos de respuestas germinativas en cuanto a la salinidad, configurando cuatro síndromes de germinación en base a los niveles de germinacíon, la velocidad y el tiempo, y la dinámica de recuperación tras la exposición a altas concentraciones de sal:

(1) Altos porcentajes de germinación a salinidades bajas-medias y germinación reducida tras exposición a altas salinidades. Este síndrome lo comparten *H. portulacoides* sin bractéolas, *S. vermiculata* sin cáliz y *A. macrostachyum*, manteniendo porcentajes medios-altos de germinación (35-99%) en concentraciones de 0,0 a 0,6 M NaCL. En cuanto a las semillas de *H. portulacoides* y *S. vermiculata* encontramos que, al compararlas con las semillas provistas de sus estructuras externas, obtenemos una mayor resistencia a la germinación a altas salinidades, lo que responde a un mecanismo de inhibición mecánica de la germinación para impedir a las semillas germinar cuando se están dispersando en el agua salada, acción que favorecen dichas estructuras (Creager, 1988; Redondo-Gómez et al., 2007).

(2) Ausencia de germinación en hipersalinidad y alta recuperación en agua dulce. Este síndrome fue compartido por *S. vermiculata* con cáliz y por *S. vera*, mostrando una baja o nula germinación en salinidades por encima de 0,3 M NaCl. Estas dos especies tienen la capacidad de mantener inactivas o latentes sus semillas durante la época de verano, cuando la salinidad aumenta, reactivando su actividad con las lluvias de primavera, las cuales lavan las sales del terreno (Álvarez-Rogel et al., 2001).

(3) Germinación alta y rápida en agua dulce tras exposición a salinidad. Esta estrategia es llevada a cabo por *H. portulacoides* y por *S. ramosissima*. La germinación de *S. ramosissima* disminuye en concentraciones por encima de 0,2 M NaCl y en *H. portulacoides* en concentraciones por encima de 0,3 M NaCl, justo las concentraciones máximas registradas en sus hábitats durante su periodo de germinación.

(4) Germinación a lo largo de un amplio rango de salinidad desde agua dulce hasta hipersalina. Este síndrome fue compartido por las tres especies de *Sarcocornia* estudiadas (*S. perennis, S. fruticosa* y *S. alpini*), permitiéndoles establecer plántulas en amplias ventanas teporales de germinación. *S. perennis* coloniza marismas bajas con gran influencia mareal (Castellanos et al., 1994), donde la salinidad muestra diferencias estacionales e interanuales, reduciendo ante esto la germinación a apartir de 0,2 M NaCl, acelerando y sincronizando la germinación cuando la salinidad decae, lo que le permite a la especie desarrollar sus plántulas rápidamente para protegerlas de las condiciones adversas de los meses más cálidos. *S. fruticosa* y *S. alpini* colonizan marismas medias-altas, ambos hábitats con grandes fluctuaciones de salinidad estacional (Álvarez-Rogel et al., 2001; Redondo-Gómez et al., 2007), donde esta especie podría germinar durante varios meses a lo largo del año e incluso en condiciones de sequía y alta salinidad, debido a su amplio rango de germinación en cuanto a la concentración de sal en el medio.

1.5 Conclusiones

1.5.1 Estrategias de dispersión de propágulos

- La mayor cantidad de los propágulos tienden a acumularse en el ecotono entre los hábitats de marisma y duna, actuando como zona sumidero. En contraste, los sedimentos arenosos adyacentes, junto con las zonas de marismas sin vegetación en el limite inferior de distribución de las mismas, fueron los hábitats con menor densidad de propágulos.
- Se describen 3 estrategias de dispersión de propágulos: (1) La dispersión a corta distancia, siendo sus propágulos mas abundantes en los hábitats fuente; (2) dispersión de media a larga distancia; y (3) dispersión mixta de las estrategias de dispersión anteriores.

1.5.2 Bancos de semillas

- La mayoría de las especies presentan bancos de semillas transitorios. Sin embargo, seis de los taxones estudiados mostraron formación de bancos de semillas persistentes, encontrando semillas intactas durante todo el año.

1.5.3 Banco de semillas de la especie anual *Salicornia ramosissima*

- La producción de semillas de *S. ramosissima* depende en mayor medida de la densidad de plantas existente en la población que de la producción de semillas por planta.
- El banco de semillas aéreo inicial tuvo mayor relevancia que el banco de semillas del suelo.

1.5.4 Dinámica poblacional *Salicornia ramosissima*

- *S. ramosissima* presenta dinámicas de población contrastadas en función de la posición fisiográfica en salinas bajo clima mediterráneo.
- Las zonas elevadas presentan menores densidades de plantas aunque con desidades más estables, debido al establecimiento de nuevas plántulas y a la mayor tasa de supervivencia de las mismas.
- Las zonas deprimidas presentan mayor densidades de plantas y de semillas en el banco de semillas, debido esto último al transporte desde zonas elevadas. También denotan mortalidad de plantas dependiente de la densidad.

1.5.5 Capacidad germinativa de las semillas de las especies halófitas en distintas concentraciones salinas

- Se observaron cuatro tipos de respuestas germinativas frente a la salinidad, (1) altos porcentajes de germinación a salinidades bajas-medias y reducción de la germinación después de exposiciones a altas salinidades; (2) ausencia de germinación en hipersalinidad y alta recuperación de geminación en agua destilada; (3) aumento de la germinación y la velocidad en agua dulce después de exposición a salinidad; (4) germinación en un amplio rango de salinidades.

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2. Artículo 1

Germination syndromes in response to salinity of Chenopodiaceae halophytes along the intertidal gradient

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Germination syndromes in response to salinity of Chenopodiaceae halophytes along the intertidal gradient

2.1 Abstract

Germination is a crucial stage in the life cycle of plants and salinity is one of the most important factors conditioning it in salt marshes. This work analyses the effects of salinity on the germination of eight dominant Chenopodiaceae species inhabiting the intertidal gradient in the Gulf of Cádiz. Seeds were collected from *Sarcocornia perennis, Atriplex portulacoides, Sarcocornia fruticosa, Salicornia ramosissima, Arthrocnemum macrostachyum, Suaeda vera, Salsola vermiculata* and *Sarcocornia alpini*. They were placed in Petri dishes at 0.0, 0.1, 0.2, 0.3, 0.6 and 0.9 M NaCl and germination was recorded during 30 days. Ungerminated seeds were transferred to distilled water and germination recovery was recorded. High salinities inhibited germination, although tended to accelerate it in the recovery assays. Beyond these common responses, the studied halophytes showed four germination syndromes: 1) high germination at low-medium salinities and reduced germination after high salinity exposure; 2) no germination at hypersalinity and high recovery germination in fresh water; 3) increased and rapid germination after salinity exposure; and 4) germinating along a wide salinity range. The two species colonising the ecotone between marshes and dunes, *Suaeda vera* and *Salsola vermiculata*, shared Syndrome 2. Syndromes 3 and 4 were shared by species colonising different habitats and Syndrome 1 by *A. macrostachyum*, and fruits of *A. portulacoides* without bracteoles and *S. vermiculata* without calyx. The germination of species from high marshes was characterized by marked responses to salinity changes, whereas the germination of low marsh species was based on low sensitivity to salinity fluctuations.

2.2 Key words

Chenopodiaceae, ecological zonation, germination recovery, mediterranean climate, salinity tolerance, salt marshes.

2.3 Introduction

Germination is a crucial stage in the life cycle of plants, especiallyfor annual species and those reproducing only by seeds (Keiffer and Ungar, 1997). In this context, germination is among the earliest processes exposed to natural selection by abiotic stress that can also activate plastic responses in parental plants increasing fitness in offspring prior to them experiencing the stressful environment (Galloway, 2005).

In salt marshes, soil salinity is one of the most important abiotic factors conditioning the germination of halophytes species. Nevertheless, seedling establishment after germination has been also described as a critical phase for plant colonization in a salty environment in spite of high germination capacities (Rubio-Casalet al., 2001). Thus, salinity organizes plant zonation through spatial-temporal gradients depending on the elevation along the intertidal frame (Egan and Ungar, 2000). Biotic interactions and other abiotic factors such as photoperiod and sediment oxygenation also change along the intertidal frame (Pennings and Callaway, 1992). Generally, most halophytes exhibit their highest germination in fresh water (Gul et al., 2013), and their germination strategies vary under high salinities depending on the species (Gul et al., 2013). Osmotic stress provoked by high salinity reduces the imbibition of water into the embryo resulting in delay or prevention of germination (Wahid et al., 1999). While some halophytes can germinate, others can be completely inhibited at high salinities (Woodell, 1985) and their germination may be increased and accelerated after a decrease in salinity (Navarro and Guitián 2003).

Species that live in highly specific habitats, such as salt marshes, often produce seeds with highly specialized adaptations (Navarroand Guitian, 2003). Thus, some halophytes have their fruits protected by permanent structures such as bracteoles and flower organs that can modulate germination in responses to salinity (Muñoz-Rodríguez et al., 2012).

Germination responses that are common to different plant species configure germination syndromes or strategies, which are useful to understand their ecological behaviours (Kettenring and Galatowitsch, 2007). Nevertheless, several studies have analyzed the germination syndromes of coexisting halophytes experimentally under saline conditions (Woodell, 1985; Song et al., 2005; Cordeiro et al., 2014). Thus, however, much is known about the general behaviour of halophyte seeds in the presence of salt (Gulet al., 2013), the relationships between halophytes' germination syndromes and their position along the intertidal gradient are still not clear for many species (Egan and Ungar 2000). In this sense, some studies have shown that salt tolerance in seed germination of coastal plants corresponded with the salinity conditions of their habitats (Callaway et al., 1990; Redondo et al., 2004).

Mediterranean tidal marsh vegetation is dominated by halo-Chenopodiaceae species, a group of plants for which most of the reproductive biology remains unknown. The colonization of the intertidal gradient by related dominant species of the same family provides a natural model system for an integrated study of germination syndromes in response to salinity for different halophytes with contrasting habitats in salt marsh ecological zonation (Redondo et al., 2004). Thus, the present work aims to analyze and compare the effects of salinity, from fresh water to hypersalinity, on the germination of *Sarcocornia perennis* (Miller) A.J. Scott, *Atriplex portulacoides* (L.) Aellen (fruits with and without bracte-oles), *Sarcocornia fruticosa* (L.) A.J. Scott, *Salicornia ramosissima* J.Woods, *Arthrocnemum macrostachyum* (Moric.) Moris, *Suaeda vera* J.F. Gmel, *Salsola vermiculata* L. (fruits with and without calyx) and *Sarcocornia alpini* (Lag.) Rivas Mart., the eight dominant Chenopodiaceae species inhabiting the intertidal gradient in the salt marshes of the Gulf of Cádiz (Southwest Iberian Peninsula). In addition, we also recorded the salinity levels in the field for every species during their germination period in order to compare this value with their germination response under laboratory controlled conditions.The eight studied species included every dominant species of this family in the Gulf of Cádiz, out of close to twenty Chenopodiaceae taxa colonising intertidal salt marshes; except for *Atriplex* *halimus* L. that dominates some high marsh areas and whose germinationwas studied previously by our research group (Muñoz-Rodríguez et al., 2012).

We hypothesized that the germination of those halophytes colonising high marshes would be more sensitive to salinity than the germination of those species living in middle and low marshes. Thus, low and middle marshes species would be able to germinate along wide salinity ranges and their germination would not be patently influenced by temporal salinity changes, in contrast with halophytes from high marshes that would show narrower germination ranges and their germination would be very sensitive to temporal changes in salinity, since high marshes experience more and higher changes in salinity conditions than lower elevations along the intertidal gradient (Callaway et al., 1990). Our results help to explain the ability of halophytes to colonise salt marshes in response to salinity and they show that some germination syndromes are shared by species colonising habitats with similar environmental conditions but others are shared by species living in contrasted habitats.

2.4 Methods

2.4.1 Studied species

Seeds were collected from the main Chenopodiaceae halophyte species colonising salt marshes along the intertidal gradient in the Gulf of Cádiz (Southwest Iberian Peninsula): *Sarcocornia perennis* in low marshes, *Atriplex portulacoides* in middle marshes, *Sarcocornia fruticosa* in middle-high marshes, *Salicornia ramosissima* in salt pans at high marshes, *Arthrocnemum macrostachyum* and *Suaeda vera* in high marshes, *Salsola vermiculata* at the ecotone between high marshes and coastal dunes, and *Sarcocornia alpini* in high marshes with fresh water penetration (See Supplementary Material).

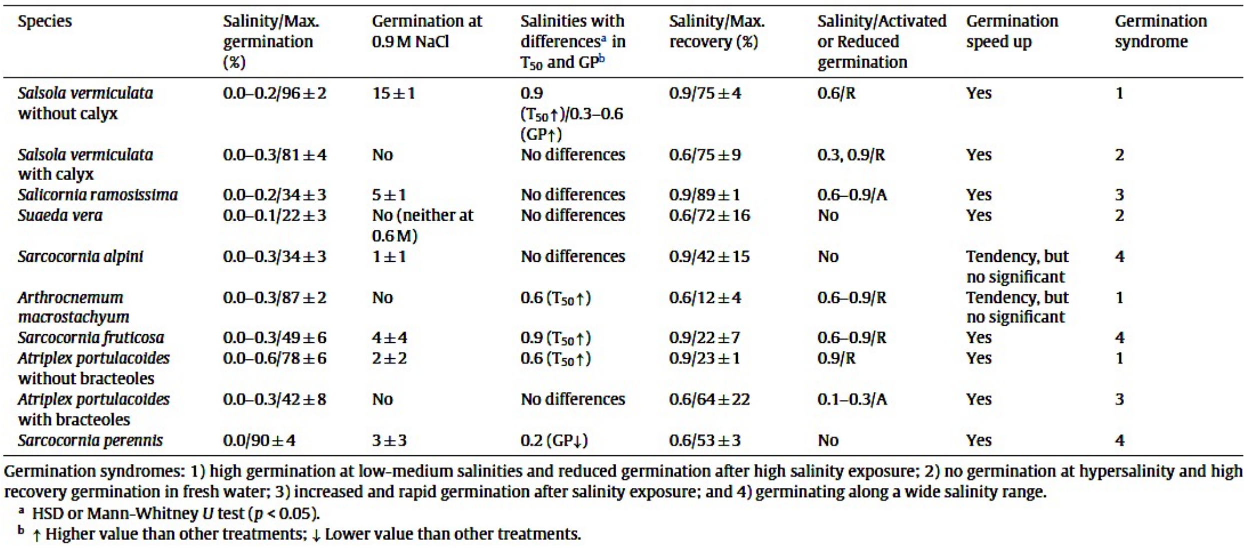
Sarcocornia perennis is a creeping perennial halophyte that occurs in well-drained Euopaean low marshes, where it disperser its ovois seeds during autumn-winter (Valdés, 1987a). *A. portulacoides* is a shrubby perennial halophyte with climbing stems, dominan ton well-drained middle salt marshes along the coasts of Europe, North Africa and Southwest Asia. It has hydrochorus propagules consisting of the fruit covered by two persistens bracteoles that are dispersed during autumn-winter (Pastor, 1987a). *S. fruticosa* is a Mediterranean perennial halophyte whit erect and multiple branches that colonizes middle-high salt marshes, dispersing its seeds also during autum-winter (Valdés, 1987a, Steffen et al., 2015). *S. ramosissima* is a anual halophyte that colonizes European and North African low, middle and hight marshes, including salt pans, where its dies during autumn-winter (Valdés, 1987b, Rubio-Casal et al., 2001). *A. macrostachyum* is a perennial halophyte whit erect branches thats grows at Mediterranean interior and coastal hight salt marshes, where it flowers and disperses its seeds during spring-summer (Gul and Khan, 1998). *S. vera* is a perennial halophyte with branchy and erect stems from coastal areas of Europe and Soutwest Asia, dispersing its seeds during spring-summer (Pastor, 1987b). *S. vermiculata* is a branchy and erect shrub widely distributed at high elevations in arid and coastal zones of South Europe, North Africa, Macaronesia and Southwest Asia, dispersing its seeds during autumn-winter covered by a persmanet calyx (Creager, 1988). *S. alpini* is a halophyte with erect branches colonising higth-intertidal zones under fresh wáter penetration in Mediterranean areas, dispersing its seeds during autumn (Valdés, 1987a; Castroviejo, 1990)

2.4.2 Plant material

The dispersal unit or propagule of each studied species was the seed covered partially and temporally by the perianth, except for *A. portulacoides* that disperses its achenes covered by two permanent bracteoles and *S. vermiculata* whose seeds are partially and permanently covered by the calyx. Mature propagules were collected from December 2010 to June 2012 (*S. perennis* – December 2010, *A. portulacoides* – January 2012, *S. fruticosa* – December 2010, *A. macrostachyum* – August 2011, *S. alpini* – December 2010, *S.vera* – June 2012, *S. ramosissima*

– December 2010, *S. vermiculata* – November 2011) from more than 10 different individuals of each species, cleaned under a magnifying glass, mixed and stored for 1–2 months in paper bags at 25°C and dark conditions until the beginning of the germination experiment. Propagules of every species were collected from the Odiel Marshes (37°08’-37°20’ N, 6°45’-7°02’ W; Spain, Southwest Iberian Peninsula; Luque et al., 2000), except for *A. macrostachyum* which was collected from the salt marshes of the Island of Tavira in the Ria Formosa (37°05’ N, 7°40’ W; Portugal, Southwest Iberian Peninsula; Costa et al., 1996).

Table 1. Main characteristics of germination in response to the salinity for the main Chenopodiaceae halophytes in the Gulf of Cádiz (Southwest Iberian Peninsula). Salinity (M NaCl) whit maximum germination rate (%), germination at 0.9 M NaCl, salinity with significant differences for T_{50} and/or germination period (GP), salinity with maximum recovery of germination (%) at fresh wáter after salinity treatment, activation (A) or reduction (R) of germination in fresh wáter after salínity treatment (Activation: total germination (salinity treatment + recovery) > germination at 0.0 NaCl; Reduction: total germination (salinity treatment + revcovery) < germination at 0.0 NaCl), germination speed up (T_{50} decrease) after hight salinity (0.6-0.9 M NaCl), and germination syndromes in response to salinity. Values are arithmetic mean ±SE (n=3)



Species	Salinity/Max. germination (%)	Germination at 0.9 M NaCl	Salinities with differences[a] in T_{50} and GP[b]	Salinity/Max. recovery (%)	Salinity/Activated or Reduced germination	Germination speed up	Germination syndrome
Salsola vermiculata without calyx	0.0–0.2/96 ± 2	15 ± 1	0.9 (T_{50}↑)/0.3–0.6 (GP↑)	0.9/75 ± 4	0.6/R	Yes	1
Salsola vermiculata with calyx	0.0–0.3/81 ± 4	No	No differences	0.6/75 ± 9	0.3, 0.9/R	Yes	2
Salicornia ramosissima	0.0–0.2/34 ± 3	5 ± 1	No differences	0.9/89 ± 1	0.6–0.9/A	Yes	3
Suaeda vera	0.0–0.1/22 ± 3	No (neither at 0.6 M)	No differences	0.6/72 ± 16	No	Yes	2
Sarcocornia alpini	0.0–0.3/34 ± 3	1 ± 1	No differences	0.9/42 ± 15	No	Tendency, but no significant	4
Arthrocnemum macrostachyum	0.0–0.3/87 ± 2	No	0.6 (T_{50}↑)	0.6/12 ± 4	0.6–0.9/R	Tendency, but no significant	1
Sarcocornia fruticosa	0.0–0.3/49 ± 6	4 ± 4	0.9 (T_{50}↑)	0.9/22 ± 7	0.6–0.9/R	Yes	4
Atriplex portulacoides without bracteoles	0.0–0.6/78 ± 6	2 ± 2	0.6 (T_{50}↑)	0.9/23 ± 1	0.9/R	Yes	1
Atriplex portulacoides with bracteoles	0.0–0.3/42 ± 8	No	No differences	0.6/64 ± 22	0.1–0.3/A	Yes	3
Sarcocornia perennis	0.0/90 ± 4	3 ± 3	0.2 (GP↓)	0.6/53 ± 3	No	Yes	4

Germination syndromes: 1) high germination at low-medium salinities and reduced germination after high salinity exposure; 2) no germination at hypersalinity and high recovery germination in fresh water; 3) increased and rapid germination after salinity exposure; and 4) germinating along a wide salinity range.

[a] HSD or Mann-Whitney *U* test ($p < 0.05$).

[b] ↑ Higher value than other treatments; ↓ Lower value than other treatments.

2.4.3 Germination experiments at salinity treatments

Before starting the experiment, germination of all taxa was tested using distilled water to ensure that they germinated without requiring any pre-treatment. All species showed regular germination rates (>25%), except *A. macrostachyum* whose germination level was lower than 1%, although no previous study described the need of any pretreatment (Pujol et al., 2000; Vicente et al., 2009). The testa of *A. macrostachyum* seeds were cut before sowing to break their dormancy and then high germination percentages were obtained (ca. 90%). Germination of *A. portulacoides* was studied with and without bracteoles and *S. vermiculata* with and without calyx, since it is known that these permanent structures affect the germination of *A. portulacoides* (Redondo et al., 2007) and *S. vermiculata* (Creager 1988).

Propagules were surface-sterilized in 70% ethanol (v/v) for 1 min, then rinsed in sterilized water and immersed in 5% (v/v) sodium hypochlorite for 10 min and, finally, rinsed three times in sterile water (Muñoz-Rodríguez et al., 2012). Next, propagules were placed in Petri dishes (9 cm diameter) with three layers of autoclaved filter paper, watered with 5 ml of different treatment solutions and sealed with adhesive tape (ParafilmTM) to avoid desiccation. Three Petri dishes with 25 seeds per dish were used per treatment and species combination. The seeds were exposed to six different concentrations of NaCl (sodium chloride puriss.pa. >99.5%, Sigma-Aldrich): 0.0 (control), 0.1, 0.2, 0.3, 0.6 and 0.9 M. This salinity range was chosen to include those salinities found along the intertidal gradient in the Southwest Iberian Peninsula, including hypersaline conditions at salt pans (Rubio-Casalet al., 2001). The experiment was carried out under controlled-environmental conditions with a 12/12 h photoperiod and 24/20°C day/night, respectively. Radiation was provided by fluorescent lamps that produced a photosynthetic photon flux density of 60 µmol $m^{-2}s^{-1}$.

The germination was recorded every two days until the 30th day, at a time when no new germination had been recorded for at least four days. A seed was considered germinated when the radicle emerged. The percentage of the 25 seeds that germinated in each plate for each treatment and the number of days necessary to reach 50% of the final germination (T50) was calculated for each plate (Muñoz-Rodríguez et al., 2012). The germination period (GP, time between first and final germination) was also recorded for each plate.

Electrical conductivity ($mScm^{-1}$) was used as a measure of sediment salinity in the field. Samples (n = 3–9 for every sampling month) were collected at every species' habitat during their main germination period, from October to March, in the Odiel Marshes and in the Ria Formosa, to compare field salinity data with the halophytes' germination responses to salinity in our lab experiments. From each sample, a mix of 5 ml of sediments and the same volume of distilled water (1:1, v:v) was placed in a tube, homogenised and the conductivity measured with a conductivity meter provided by Crison Instruments 5064 (Hach Lane Spain S.L.U., Barcelona, Spain)(Nieva et al., 2001).

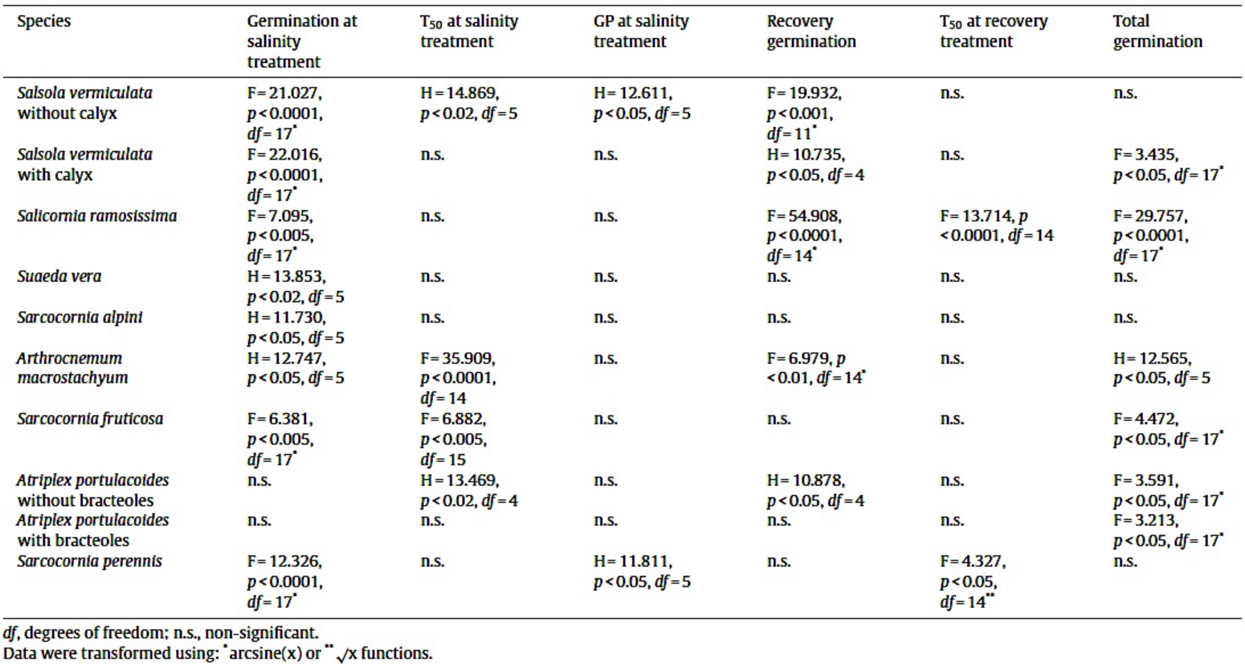
2.4.4 Recovery of germination after salinity treatments

The seeds that did not germinate after the salinity treatment were cleaned with distilled water, sterilized again and sowed indistilled water. The germination was recorded for another 30 days, and the recovery of germination was calculated as the percentage of the initial 25 seeds that did not germinate in each salt treatment but germinated in the recovery experiment (quiescent seeds) (Baskinand Baskin, 1985). The total germination for each treatment was calculated as the sum of germinations at salinity and recovery treatments. It was considered that salinity activated germination ('salt stimulation' following Woodell, 1985) when the total germination was higher than the germination of the control treatment.

2.4.5 Data analysis

Deviation to the mean was calculated as Standard Error (SE). Statistical analyses were carried out using Statistica 8.0 (StatSoft Inc.,USA). Data were tested for homogeneity of variance and normality with the Levene test and the Kolmogorov-Smirnov Test, respectively ($p < 0.05$). Differences between means of germination, T50 and GP for different salinity treatments were analyzed using one-way analysis of variance (ANOVA) with the salinity treatment as the grouping factor (F-test). Tukey's Honest Significant Difference (HSD) test between two means was calculated only if the F-test was significant at the 0.05 level of probability. When homogeneity of variance between groups was not found, data were transformed using $\sqrt{x}$ or arcsine (x) functions. If homogeneity of variance was not achieved by data transformation, then the means were compared using Kruskal-Wallis H-test and Mann-Whitney U test.

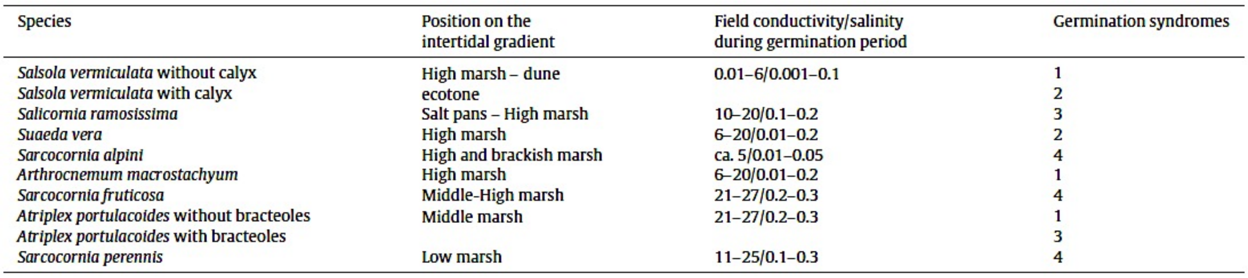
Table 2. Statistical results (one-way analisis of variance ANOVA (F) or parametric Kruskal-Wallis test (H)) comparing salinity treatmenst for main germination responses of the dominant Chenopodiaceae halophytes in the Gulf of Cádiz (Southwest Iberian Peninsula).



Species	Germination at salinity treatment	T_{50} at salinity treatment	GP at salinity treatment	Recovery germination	T_{50} at recovery treatment	Total germination
Salsola vermiculata without calyx	F = 21.027, $p < 0.0001$, $df = 17^{*}$	H = 14.869, $p < 0.02$, $df = 5$	H = 12.611, $p < 0.05$, $df = 5$	F = 19.932, $p < 0.001$, $df = 11^{*}$	n.s.	n.s.
Salsola vermiculata with calyx	F = 22.016, $p < 0.0001$, $df = 17^{*}$	n.s.	n.s.	H = 10.735, $p < 0.05$, $df = 4$	n.s.	F = 3.435, $p < 0.05$, $df = 17^{*}$
Salicornia ramosissima	F = 7.095, $p < 0.005$, $df = 17^{*}$	n.s.	n.s.	F = 54.908, $p < 0.0001$, $df = 14^{*}$	F = 13.714, $p < 0.0001$, $df = 14$	F = 29.757, $p < 0.0001$, $df = 17^{*}$
Suaeda vera	H = 13.853, $p < 0.02$, $df = 5$	n.s.	n.s.	n.s.	n.s.	n.s.
Sarcocornia alpini	H = 11.730, $p < 0.05$, $df = 5$	n.s.	n.s.	n.s.	n.s.	n.s.
Arthrocnemum macrostachyum	H = 12.747, $p < 0.05$, $df = 5$	F = 35.909, $p < 0.0001$, $df = 14$	n.s.	F = 6.979, $p < 0.01$, $df = 14^{*}$	n.s.	H = 12.565, $p < 0.05$, $df = 5$
Sarcocornia fruticosa	F = 6.381, $p < 0.005$, $df = 17^{*}$	F = 6.882, $p < 0.005$, $df = 15$	n.s.	n.s.	n.s.	F = 4.472, $p < 0.05$, $df = 17^{*}$
Atriplex portulacoides without bracteoles	n.s.	H = 13.469, $p < 0.02$, $df = 4$	n.s.	H = 10.878, $p < 0.05$, $df = 4$	n.s.	F = 3.591, $p < 0.05$, $df = 17^{*}$
Atriplex portulacoides with bracteoles	n.s.	n.s.	n.s.	n.s.	n.s.	F = 3.213, $p < 0.05$, $df = 17^{*}$
Sarcocornia perennis	F = 12.326, $p < 0.0001$, $df = 17^{*}$	n.s.	H = 11.811, $p < 0.05$, $df = 5$	n.s.	F = 4.327, $p < 0.05$, $df = 14^{**}$	n.s.

df, degrees of freedom; n.s., non-significant.
Data were transformed using: *arcsine(x) or $^{**}\sqrt{x}$ functions.

Table 3. Position on the intertidal gradient, range of electrical conductivity (mScm^{-1}) of the interstitial water of the sediment, as a measure of salinity (M NaCl), during their germination period (autum-winter) in natural habits, and germination síndromes in response to salinity (see Table 1) for the main Chenopodiaceae halophytes in the Gulf of Cádiz (Southwet Iberian Peninsula)



Species	Position on the intertidal gradient	Field conductivity/salinity during germination period	Germination syndromes
Salsola vermiculata without calyx	High marsh – dune ecotone	0.01–6/0.001–0.1	1
Salsola vermiculata with calyx			2
Salicornia ramosissima	Salt pans – High marsh	10–20/0.1–0.2	3
Suaeda vera	High marsh	6–20/0.01–0.2	2
Sarcocornia alpini	High and brackish marsh	ca. 5/0.01–0.05	4
Arthrocnemum macrostachyum	High marsh	6–20/0.01–0.2	1
Sarcocornia fruticosa	Middle-High marsh	21–27/0.2–0.3	4
Atriplex portulacoides without bracteoles	Middle marsh	21–27/0.2–0.3	1
Atriplex portulacoides with bracteoles			3
Sarcocornia perennis	Low marsh	11–25/0.1–0.3	4

Germination syndromes were analyzed applying cluster analysis, based on Euclidean distances and the single linkage as amalgamation rule, to a matrix with halophytes species (at rows) and the main germination and recovery traits (at columns) (highersalinity with germination, maximum final germination, final germination at 0.6 and 0.9 M NaCl, salinity with T50 increase, maximum recovery germination, activated or reduced (1-0) germination and germination speed up (1-0) after salinity exposure). The data matrix was standardized prior to analysis.

2.5 Results

2.5.1 Germination at salinity treatments

Every taxon showed its maximum germination at lower salinities. *S. perennis*, *S. fruticosa, A. portulacoides* without bracteoles, *A.macrostachyum* and *S. vermiculata* with and without calyx showed higher maximum germination (>70%) than *S. ramosissima, A. portulacoides* with bracteoles, *S. alpini* and *S. vera* (<42%) (0.0 M NaCl between species: Kruskal-Wallis test, H = 21.874, p < 0.01, n = 30; Mann-Whitney U test, p < 0.05) (Table 1, Fig. 1).

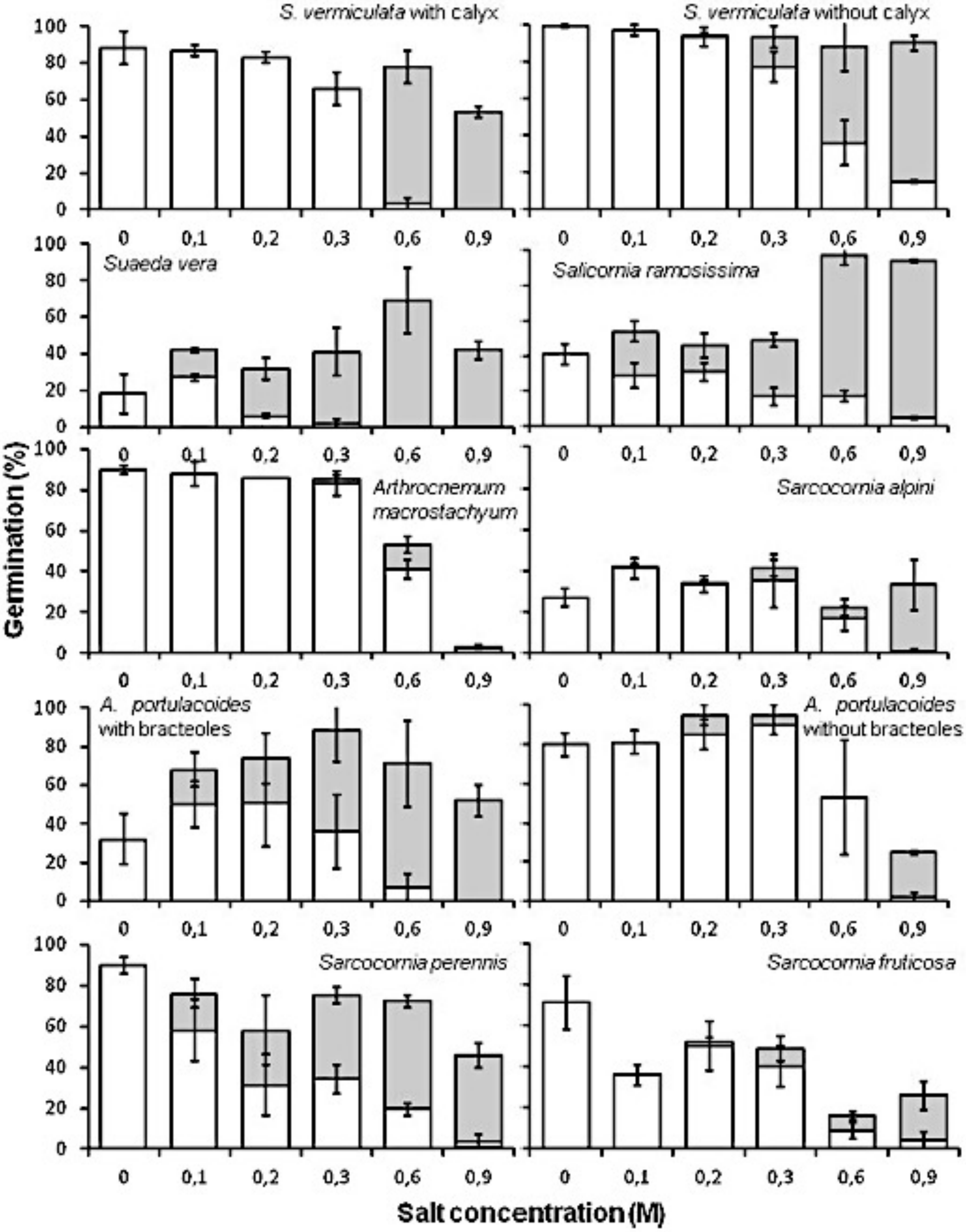


Figure 1. Germination in salt treatments (0.0, 0.1, 0.2, 0.3, 0.6 and 0.9 M NaCl) (White) and recovery germination at 0.0 M after salt treatment (grey) for the eight main Chenopodiaceae halophytes in the Gulf of Cádiz (Southwest Iberian Peninsula) (aritmetic mean ± SE, n=3)

A. *portulacoides* without bracteoles was less sensitive to salinity than the other species, which was reflected in the maintenance of high germination percentages from 0.0 to 0.6 M NaCl (with a mean of 78 ± 6%) and in being able to germinate even at 0.9 M NaCl (2 ± 2%) (Table 1, Fig. 1). Salinity slowed down its germination (higher T50) only at 0.6 M NaCl (Table 1, Mann-

Whitney U test, $p < 0.05$), without showing significant differences in GP (Tables 1 and 2, Fig. 2).

The second most tolerant species to salinity was *S. vermiculata* without calyx, showing high germination percentages between 0.0 and 0.3 M NaCl (>75%) and the highest germination for every species at hypersalinity conditions (15 ± 1%) (Table 1, Mann-Whitney U test, $p < 0.05$) (Table 1, Fig. 1). It slowed down its T50 only at the highest salinity (14 ± 4 days) in relation to lowest (1 ± 0 days) (Table 1, Mann-Whitney U test, $p < 0.05$), increasing its GP at 0.3–0.6 M NaCl (Table 1, Mann-Whitney U test, $p < 0.05$) (Fig. 2).

The third most tolerant species to salinity was a group of 5 species (*S. fruticosa, A. portulacoides* with bracteoles, *A. macrostachyum, S. alpini and S. vermiculata* with calyx) with a constant germination from 0.0 to 0.3 M NaCl. Out of these species, *S. alpini* showed the highest germination at 0.6 M NaCl in relation to its maximum germination (36 ± 12% of maximum germination) and *S. vermiculata* with calyx the lowest value (only 3 ± 3% of maximum germination). In addition, *S. alpini* and *S. fruticosa* were the only species of this group able to germinate at hypersalinity (Table 1, Fig. 1). Most of these species did not show significant differences in T50 and GP among treatments (Table 2). Their T50 was always between 5 and 11 days except for *A. portulacoides* with bracteoles with T50 values between 14-22 days. *S. fruticosa* and *A. macrostachyum* showed their highest T50 (>15 days) at the highest salinity (Table 1, HSD-test, $p < 0.05$) (Fig. 2).

Salicornia ramosissima and *S. perennis* were able to germinateat the highest salinity levels, but their final germination decreased markedly even at low salinities. *S. ramosissima* was able to keep germination percentages without showing significant decreaseonly until 0.2 M NaCl (34 ± 3%) (Table 2, Mann-Whitney U test,$p < 0.05$), with T50 around 10 days and a GP ca. 20 days for every treatment (Table 1, Figs. 1 and 2). On the other hand, *S. perennis* showed its highest germination in the absence of salt (90 ± 4%), decreasing gradually and significantly from 0.1 M NaCl (Table 2,Mann-Whitney U test, $p < 0.05$) (Fig. 1), without showing a clearand significant increase in T50 with salinity and lowering its GP at 0.2 M Na Cl (Table 1, Mann-Whitney U test, $p < 0.05$) (Table 1, Fig. 2).

Suaeda vera presented low germination at all salinities (<20%), being the most sensitive species to salinity, which was reflected on the reduction of its germination from 0.2 M NaCl compared with 0.0 M NaCl (Table 2, Mann-Whitney U test, $p < 0.05$) and because it was the only species that was not able to germinate at the two highest salinities (Fig. 1). Its mean T50 values were between 8 and 16 days and its GP was ca. 10 days, without significant differences between treatments (Tables 1 and 2 Fig. 2).

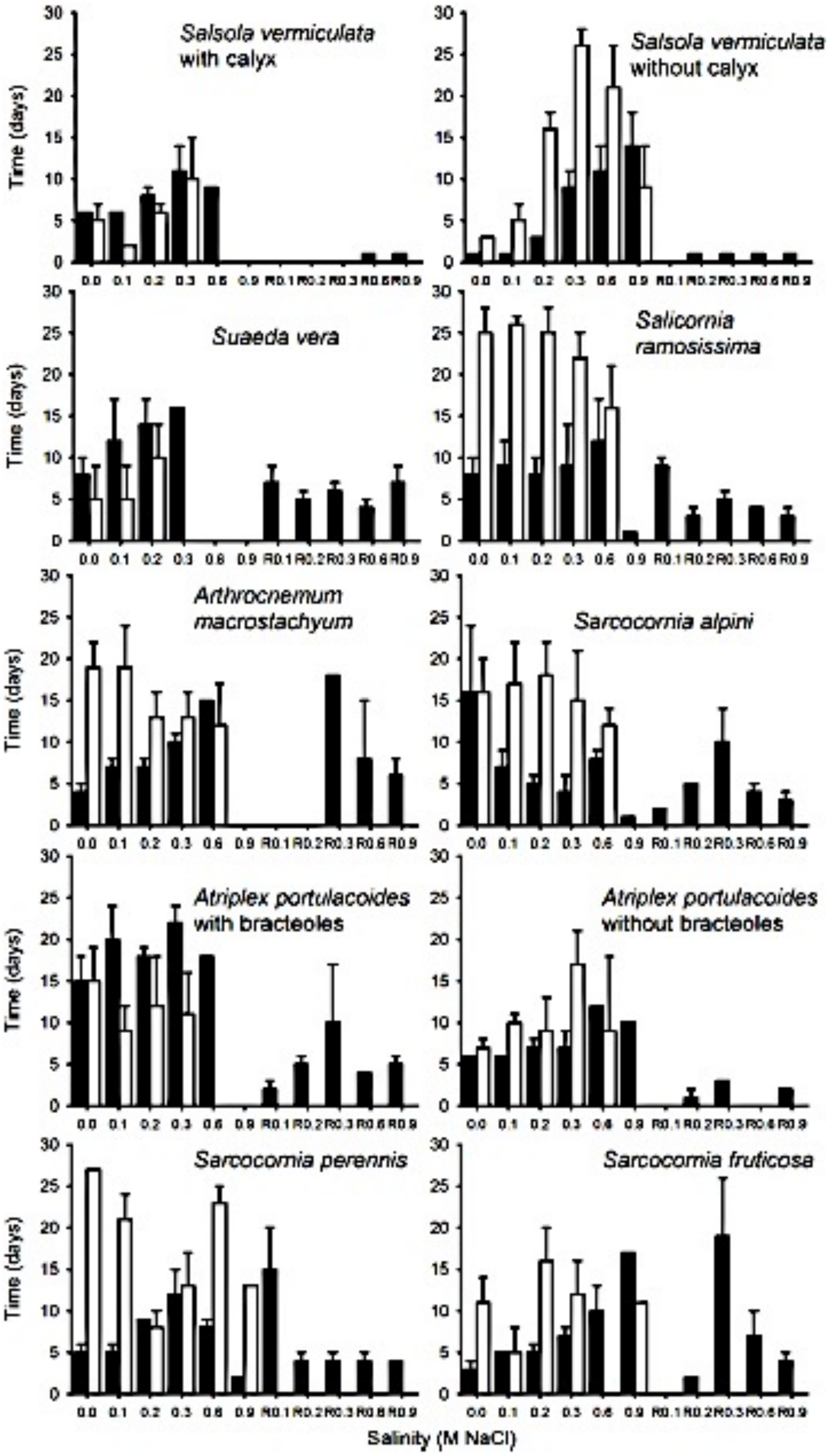


Figure 2. Niumber of days to reach 50% of the final germination (T_{50}) (black bars) and germination period (GP = number of days to final germination - number of days to first germination) (white bars) in salt treatments and for the eight main Chenopodiaceae halophytes in te Gulf of Cádiz (Southwest Iberian Peninsula) (aritmetic mean ±SE, n = 1-3).

S. vermiculata colonised the less salty sediments during its germination period, followed by *S. alpini, S. vera*, *A. macrostachyum* and *S. ramosissima*. The three species colonising the lower elevations on the intertidal gradient, *S. fruticosa, A. portulacoides* and *S.perennis*, were growing at the saltiest sediments during their germination period, however salinity was always lower than 0.3 M NaCl (Table 3).

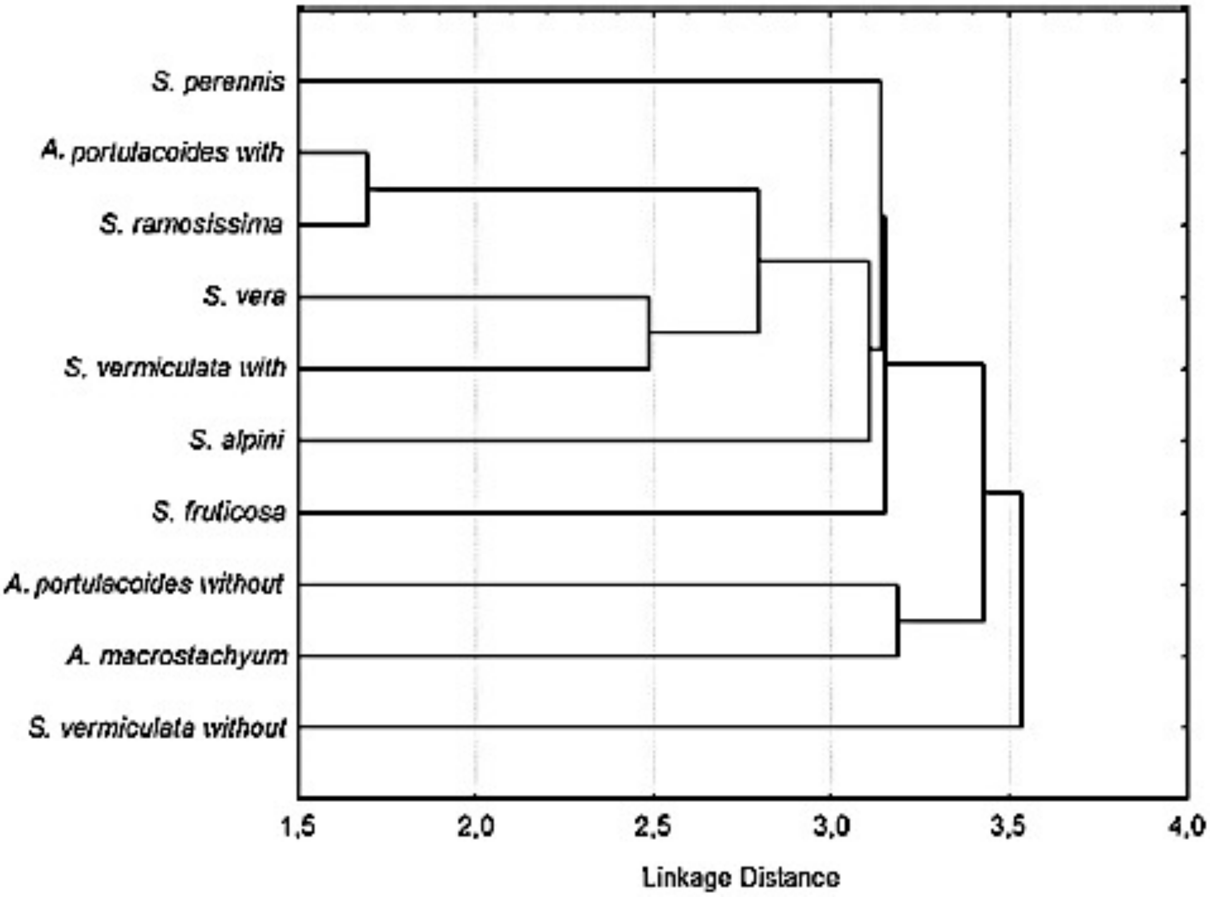


Figure 3. Cluster analysis (base don Euclidean distances) using germination traits under salinity tratments and its recovery after salinity for the eight main Chenopodiaceae halophytes along the intertidal gradient in salt marshes of the Gulf of Cádiz (Southwest Iberian Peninsula)

2.5.2 Germination recovery

The number of quiescent seeds increased with salinity for every species, except for *S. perennis, A. portulacoides* with bracteoles, *S. fruticosa* and *S. alpini* (Table 2, Fig. 1). At the same time, germination tended to speed up (lower T50) after salinity exposure, especially for higher salinities, however these differences were not significant for *A. macrostachyum* and *S. alpini* (Table 1, Fig. 2).

A. portulacoides without bracteoles , *S. fruticosa, A. macrostachyum* and *S. alpini* showed the lowest germination recovery values (<35%). The total germination (treatments + recovery) of *S. fruticosa* and *A. macrostachyum* was lower at higher salinities than at the control treatment (Table 2, Mann-Whitney U test,$p < 0.05$). In the case of *A. macrostachyum*, the total germination at 0.9 M NaCl was as low as 3 ± 1%. In contrast, *S. ramosissima* showed higher total germination at medium and high salinities, respectively, than at the control treatment (Table 2, Mann-Whitney Utest, $p < 0.05$; Fig. 1). *S. perennis, A. portulacoides* without bracteoles, *S. alpini, S. vera* and *S. vermiculata* with and without calyx showed similar total germination at every salinity than at the control treatment, except for *S. vermiculata* without calyx at 0.6 M that showed a slight but significant decrease (Tables 1 and 2, Fig. 1).

2.5.3 Germination syndromes

Cluster analysis using main germination traits for salinity treatments and for recovery after salinity (listed in Table 1) grouped *Atriplex portulacoides* with bracteoles and *Salicornia ramosissima* as those species with more similar germination responses (linkage distance of 1.65 over 3.55), based on the lack of any timing response at the first salinity exposure and the activation of rapid recovered germination after medium-high salinities (Table 1, Fig. 3). *Suaeda vera* and *Salsola vermiculata* with calyx were grouped together (linkage distance 2.49/3.55), and with the former pair of species (linkage distance 2.80/3.55), based on showing none germination at hypersalinity conditions, none timing response at first salinity exposure, and high and rapid germination recovery after salinity exposure (Table 1, Fig. 3).

The three species of *Sarcocornia (S. alpini, S. fruticosa* and *S. perennis)* were clustered with the two former pairs of species, but with alow linkage distance (3.16 over 3.55), based on germinating along a wide salinity range from fresh water to hypersalinity (Table 1,Fig. 3).

On the other hand, *A. portulacoides* without bracteoles and *Arthrocnemum macrostachyum* were grouped together just with a linkage distance of just 3.24 over 3.55, and *Salsola vermiculata* without calyx showed the most different germination responses in relation to other species. These three species shared high germination percentages at low-medium salinities, slowing down their germination at high salinities, and reduced germination after highsalinity exposure (Table 1, Fig. 3).

2.6 Discussion

An increase in salinity, from distilled water to hypersalinity, inhibited germination and enforced seed quiescence for every species, as reported previously for different Chenopodiaceae taxa (e.g. Pujol et al., 2001; Singh et al., 2014). The prevention of germination at high salinities allows halophytes to avoid stressful conditions for seedlings by remaining quiescent or dormant at highsalinity (Song et al., 2005).

On the other hand, germination tended to be accelerated (lower T50) in fresh water after salinity exposure for every species. This response may allow a quick germination of the studied species just following rainfall episodes, occupying the available space rapider than other species (Navarro and Guitián, 2003). Except *A. macrostachyum* and *S. vera* that disperse their seeds in spring, the dispersion of every studied species takes place during autumn-winter so their germination would be accelerated after salinity exposure and the salt washout by rainfalls during winter-spring and after autumn rainfalls when their seeds passed their first summer (drought season at Mediterranean marshes when salinity is the highest). This feature has been reported previously for many halophytes (Pujol et al., 2001; Redondo et al., 2004).

Beyond the above-mentioned common responses, the main Chenopodiaceae halophytes at the Gulf of Cádiz showed very diverse germination responses to salinity, configuring four germination syndromes based on germination levels, speed and timing, and the dynamic of germination recovery after salt exposure: 1) high germination percentages at low-medium salinities and reduced germination after high salinity exposure; 2) no germination at hypersalinity and high germination recovery at fresh water; 3) increased and rapid germination at fresh water after salinity exposure; and 4) germination along a wide salinity range from fresh water to hypersalinity.

Despite colonising habitats with contrasted salinities during their germination period, *A. portulacoides* (fruits covered by bracteoles when dispersing) and *S. ramosissima* shared a germination syndrome based merely on the activation of a rapider germination after the exposure to medium-high salinities. *S. ramosissima* germination decreased at salinities higher than 0.2 M NaCl and *A. portulacoides'* germination decreased at 0.3 M NaCl, just at the maximum salinities recorded in their habitats during their germination period. This germination strategy would allow them to effectively occupy a wide range of habitats along the intertidal gradient (Bouchard and Lefeuvre, 1996; Rubio-Casal et al., 2001).

High seedling production during 'windows of germination' (Noeand Zedler, 2001) seems to be especially important for those halophytes whose seedlings are less salt tolerant, suffering a decoupling between optimum ranges of salinity during germination, seedling and adult stages (Woodell, 1985). Callaway et al. (1990) found that the germination responses to salinity of annual species, such as *S. ramosissima*, corresponded with the winter and spring soil salinities of the zones they occupy in the field. Rubio-Casal et al. (2003) working also *on S. ramosissima* in the Odiel Marshes found very different responses compared to our results (higher germination levels and no stimulation of germination after salinity exposure), pointing to the existence of contrasting inter-population differences in response to salinity as reported for other species (Cordeiroet al., 2014).

Suaeda vera and *S. vermiculata* (with dispersed fruits covered by the calyx) shared a germination syndrome based on none or very low germination at high salinities (> 0.3 M NaCl) and high percentages of rapid germination when salinity decreased. *S. vera* showed a reduced germination from 0.2 M NaCl, just at the maximum salinity recorded in the field during its germination period, while *S. vermiculata* was able to germinate at much higher salinities than those recorded at its habitat in autumn-winter. These two species colonise the upper distribution limit of salt marshes where salinity was low during their germination period. Their seeds would remain quiescent or dormant during the summer drought when salinity increases and their germination would be associated with salinity decreases after summer time due to direct washing of salts by rainfall and by run-off coming from slopes (usually from adjacent coastal dunes) (Álvarez-Rogel et al., 2001).

The germination of *S. perennis* and *S. fruticosa* droped markedly at salinities higher than 0.3 M NaCl which was the maximum salinity recorded at their habitats during their germination period. The germination responses of the three studied *Sarcocornia species* (*S. perennis, S. fruticosa* and *S. alpini*) was characterized essentially by germinating at a wide salinity range (from fresh water to hypersalinity), which would enable them to set seedlings in broad spatial-temporal windows of germination. *S. perennis* colonizes low-middle marshes under high tidal influence (Castellanos et al.,1994), where salinity shows seasonal and inter-annual differences, however, these seasonal changes are lower than at upper elevations in the tidal frame (Callaway et al., 1990). In addition, *S. perennis* reduced its GP at 0.2 M NaCl, accelerating and synchronizing its germination, which was also recorded by Redondo et al. (2004). A rapid and synchronized germination when salinity drops during the rainy season may allow *S. perennis* to develop most of its seedling squickly enough to protect them through the hottest months when its mortality is the highest (Kaminsky et al., 2015). On the other hand, *S. fruticosa* and *S. alpini* colonise middle-high salt marshes and brackish marshes, respectively, both habitats with high seasonal salinity fluctuations (Álvarez-Rogel et al., 2001; Redondo-Gómezet al., 2007), where these species would be able to germinate during several months throughout the year and even in years of drought and high salinities. Germinating even at high salinities may reduce interspecific competition and exposure their seedlings to abiotic stressful conditions, but these species are very tolerant to salinity (Redondo-Gómez et al., 2006). Redondo et al. (2004) working on a *S. perennis* and *S. fruticosa* populations also found high final germination at low salinities (ca. 85%) and germination even at hypersaline conditions for both species.

Fruits of *A. portulacoides'* without bracteoles, *S. vermiculata* without calyx and *A. macrostachyum* were the most tolerant to the first exposure to salinity (maintaining medium-high germination percentages (35–99%) from 0.0 to 0.6 M NaCl, and the two former species being able to germinate even at hypersalinity conditions). They shared a germination syndrome based on high germination levels at low-medium salinities (>80%), slow germination at high salinities and reduced germination after high salinity exposure. As in our study, previous works also found lower and slower germination when *A. portulacoides* and *S. vermiculata* fruits were covered with bracteoles and calyx, respectively, than when they were devoid of these permanent structures.

These responses were especially significant at high salinities, enforcing seed dormancy by mechanical inhibition of germination (Creager, 1988; Redondo-Gómez et al., 2007). Such enforced dormancy could prevent germination during the dispersal in seawater that is favoured by bracteoles and calyx (Ungar and Khan, 2001). Reduced germination recorded for *A. portulacoides* and *S. vermiculata* in the recovery experiment at high salinities could reflect high seed mortality due to deleterious salinity effects that would be avoided thanks to bracteoles and calyx, respectively. On the other hand, delays of germination and seed dormancy at high salinities have also been previously found for *A. macrostachyum* (Vicente et al., 2009). The seeds of every halophyte in our study have a membranous testa, except those of *A. macrostachyum* and *S. vera*, the only studied species that disperse their seeds in spring covered with a coriaceous testa; besides *A. portulacoides* and *S. vermiculata* that have their fruits covered by permanent protecting structures (Pastor,1987a,b; Valdés, 1987a,b). Having a coriaceous testa or fruit protecting structures would allow *A. macrostachyum* and *S. vera* to establish permanent seed banks waiting for adequate environmental conditions to germinate, like those recorded at their habitats during autumn-winter.

In general, our results showed that species growing in high marshes (*A. macrostachyum, S. vera, S. ramosissima* from salt pansand *S. vermiculata*), where inter- and intra-annual salinity changes were patent, showed germination syndromes characterized by marked response to temporal salinity changes, evidenced in the recovery experiment after salt exposure. On the opposite extreme of the intertidal gradient, *S. perennis*, a species growing in low marshes with narrow salinity fluctuations, showed a germination syndrome based on low sensitivity to salinity changes. Finally, those species colonising middle/high marshes (*A. portulacoides, S.fruticosa* and *S. alpini*) presented two distinct germination syndromes shared also by species from low and high marshes. This seemed to be related with some syndromes having very versatile germination strategies and with the influence of phylogeny for the three closely related *Sarcocornia* species.

2.7 Conclusions

The dominant Chenopodiaceae halophytes in the salt marshes of the Gulf of Cádiz (Southwest Iberian Peninsula) showed contrasting germination responses to salinity, which were in accordance with the salinity regimes at both extremes of the intertidal gradient, in both low and high marshes. Nevertheless, species from middle marshes did not share a clear and unique germination syndrome. In contrast, the protecting structures of *A. portulacoides* and *S. vermiculata*, which are not normally lost in the field, changed their germination strategies completely in comparison with unprotected fruits.

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3. Artículo 2

Contrasting propagule dispersal and halophyte seed banks along the intertidal gradient

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

3.1 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.

3.2 Key words

Tidal salt marsh, plant zonation, dispersal, persistent seed bank, Ría Formosa.

3.3 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 colonizing 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.

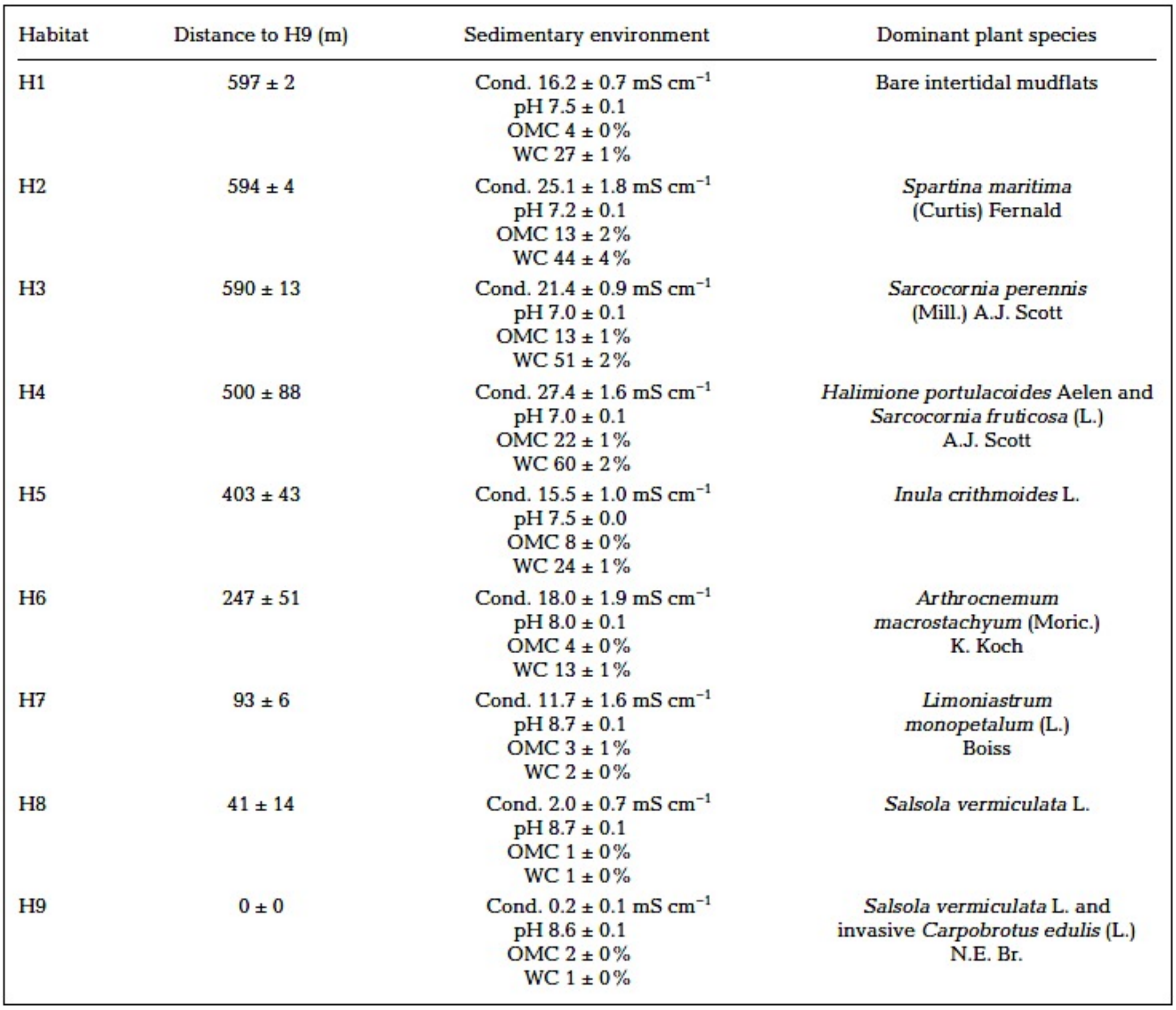
Previous studies have described the sedimentary environment, vegetation zonation and germination responses of halophytes to salinity along the intertidal gradient in Ría Formosa Natural Park (south- west Iberian Peninsula) (Contreras-Cruzado et al. 2017, Muñoz-Rodríguez et al. 2017). The detailed knowledge 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.

3.4 Material and methods

3.4.1 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^2, of which 48 km^2 are covered by salt marshes (Teixeira & Alvim 1978) (Fig. S1 in the Supplement at 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 finegrained alluvial sediments with sediment accretion rates ca. 8−9 mm yr^{-1} (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 contect [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 recordered by Contretas-Cruzado et al. (2017). Values are mean ± SE (n=3-9)



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

3.4.2 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°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 ± 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 g l– 1) and sodium bicarbonate (25 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 × 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 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 $habitat^{-1}$. 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; insquares of 10 × 10m, n = 3 plots $habitat^{-1}$) 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.

3.4.3 Statystical analysis

Statistics were carried out with STATISTICA 8.0 (Statsoft), applying a significance level of $\alpha \leq 0.05$. Desviation 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 (ρ).

3.5 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 ± 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).

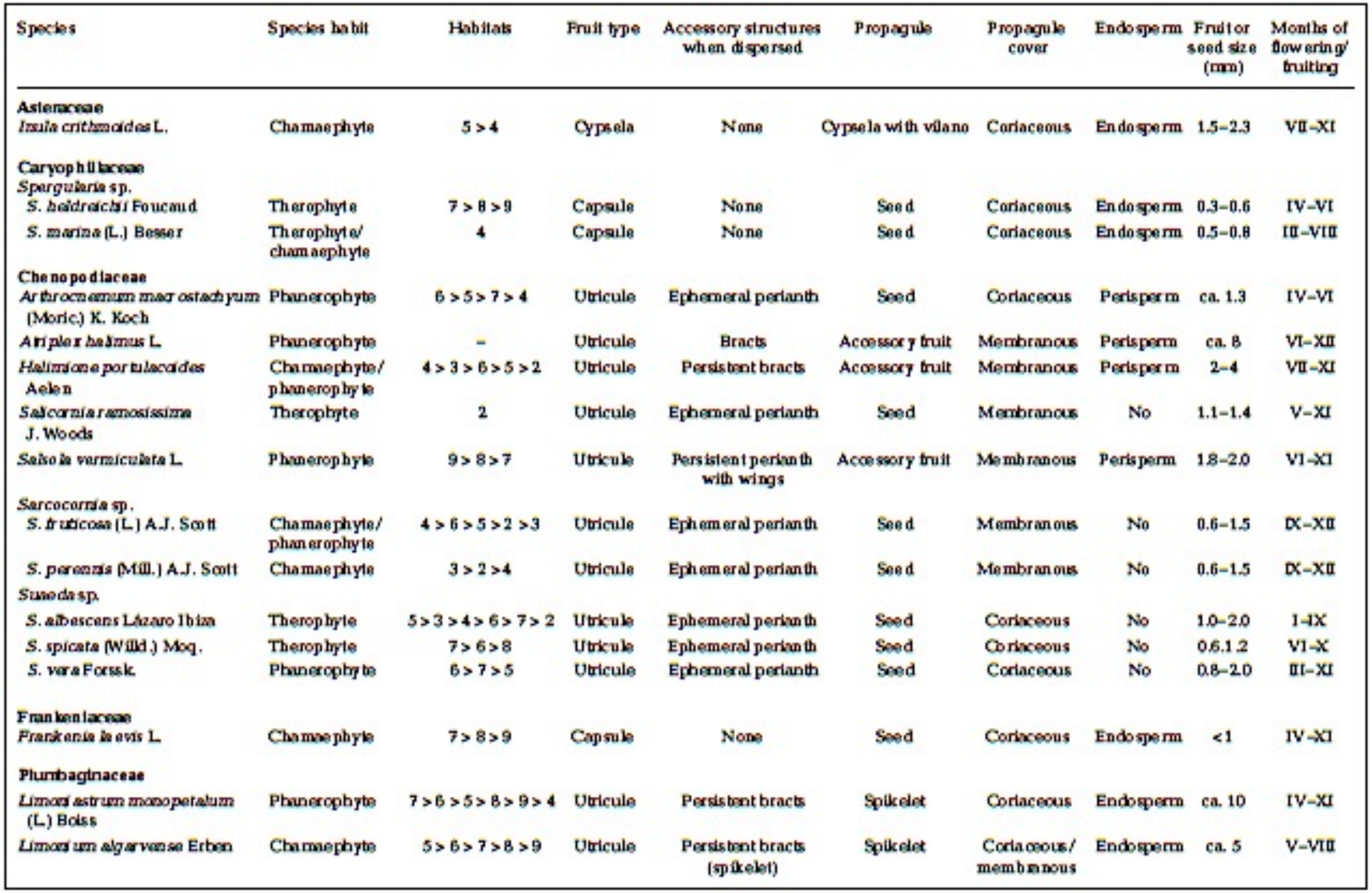
The propagules of 7 halophyte species (*Inula crithmoides*, *Frankenia laevis*, *Polygonum equisetiforme, Puccinellia convoluta, Atriplex halimus* [not present in the habitats studied but colonizing nearby high marshes], *Limonium narbonense* and *Spartina maritima*) were very scarce in the sediments, 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. 3 A,C). The propagules of *H. portulacoides* were more abundant at H4 and H6 (H8,420 =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 (H4,420 = 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 (H8,420 = 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.* were more abundant at H8 and H9 ($H_{8,420} = 18.89$, $p < 0.05$) (Fig. 4K), coinciding with higher cover from *S. marina* ($\rho = +0.097$,

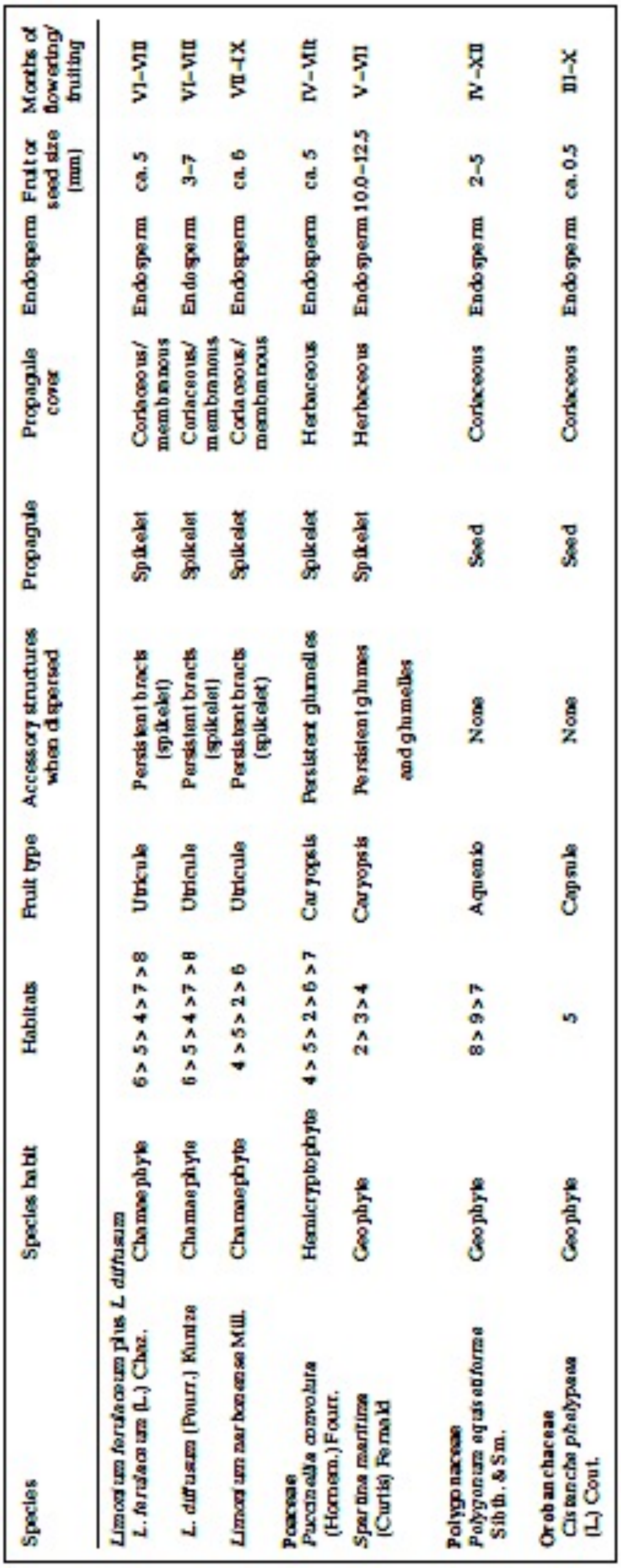
Table 2. Families and scientisc name, species habit, coloniced hábitats and seed, fruits and propagules characteristics for plant species recorded in the seed bank of salt marshes distributed along the intertidal gradient at

Ria Formosa (southwest Iberian Peninsula). Data sources: A.F. Muñoz-Rodriguez (pers. obs.), Castroviejo (1986-2015), Valdés et al. (1987) and Fernández-Illescas et al. (2010). -: taxón not found in the study área 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
Asteraceae									
Inula crithmoides L.	Chamaephyte	5 > 4	Cypsela	None	Cypsela with vilano	Coriaceous	Endosperm	1.5–2.3	VII–XI
Caryophillaceae									
Spergularia sp.									
S. heldreichii Foucaud	Therophyte	7 > 8 > 9	Capsule	None	Seed	Coriaceous	Endosperm	0.3–0.6	IV–VI
S. marina (L.) Besser	Therophyte/ chamaephyte	4	Capsule	None	Seed	Coriaceous	Endosperm	0.5–0.8	III–VIII
Chenopodiaceae									
Arthrocnemum macrostachyum (Moric.) K. Koch	Phanerophyte	6 > 5 > 7 > 4	Utricule	Ephemeral perianth	Seed	Coriaceous	Perisperm	ca. 1.3	IV–VI
Atriplex halimus L.	Phanerophyte	–	Utricule	Bracts	Accessory fruit	Membranous	Perisperm	ca. 8	VI–XII
Halimione portulacoides Aelen	Chamaephyte/ phanerophyte	4 > 3 > 6 > 5 > 2	Utricule	Persistent bracts	Accessory fruit	Membranous	Perisperm	2–4	VII–XI
Salicornia ramosissima J. Woods	Therophyte	2	Utricule	Ephemeral perianth	Seed	Membranous	No	1.1–1.4	V–XI
Salsola vermiculata L.	Phanerophyte	9 > 8 > 7	Utricule	Persistent perianth with wings	Accessory fruit	Membranous	Perisperm	1.8–2.0	VI–XI
Sarcocornia sp.									
S. fruticosa (L.) A.J. Scott	Chamaephyte/ phanerophyte	4 > 6 > 5 > 2 >3	Utricule	Ephemeral perianth	Seed	Membranous	No	0.6–1.5	IX–XII
S. perennis (Mill.) A.J. Scott	Chamaephyte	3 > 2 >4	Utricule	Ephemeral perianth	Seed	Membranous	No	0.6–1.5	IX–XII
Suaeda sp.									
S. albescens Lázaro Ibiza	Therophyte	5 > 3 > 4 > 6 > 7 > 2	Utricule	Ephemeral perianth	Seed	Coriaceous	No	1.0–2.0	I–IX
S. spicata (Willd.) Moq.	Therophyte	7 > 6 > 8	Utricule	Ephemeral perianth	Seed	Coriaceous	No	0.6.1.2	VI–X
S. vera Forssk.	Phanerophyte	6 > 7 > 5	Utricule	Ephemeral perianth	Seed	Coriaceous	No	0.8–2.0	III–XI
Frankeniaceae									
Frankenia laevis L.	Chamaephyte	7 > 8 > 9	Capsule	None	Seed	Coriaceous	Endosperm	<1	IV–XI
Plumbaginaceae									
Limoniastrum monopetalum (L.) Boiss	Phanerophyte	7 > 6 > 5 > 8 > 9 > 4	Utricule	Persistent bracts	Spikelet	Coriaceous	Endosperm	ca. 10	IV–XI
Limonium algarvense Erben	Chamaephyte	5 > 6 > 7 > 8 > 9	Utricule	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
Limonium ferulaceum plus *L. diffusum*									
L. ferulaceum (L.) Chaz.	Chamaephyte	6 > 5 > 4 > 7 > 8	Utricule	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	ca. 5	VI–VIII
L. diffusum (Pourr.) Kuntze	Chamaephyte	6 > 5 > 4 > 7 > 8	Utricule	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	3–7	VI–VIII
Limonium narbonense Mill.	Chamaephyte	4 > 5 > 2 > 6	Utricule	Persistent bracts (spikelet)	Spikelet	Coriaceous/ membranous	Endosperm	ca. 6	VII–IX
Poaceae									
Puccinellia convoluta (Hornem.) Fourr.	Hemicryptophyte	4 > 5 > 2 > 6 > 7	Caryopsis	Persistent glumelles	Spikelet	Herbaceous	Endosperm	ca. 5	IV–VIII
Spartina maritima (Curtis) Fernald	Geophyte	2 > 3 > 4	Caryopsis	Persistent glumes and glumelles	Spikelet	Herbaceous	Endosperm	10.0–12.5	V–VII
Polygonaceae									
Polygonum equisetiforme Sibth. & Sm.	Geophyte	8 > 9 > 7	Aquenio	None	Seed	Coriaceous	Endosperm	2–5	IV–XII
Orobanchaceae									
Cistanche phelypaea (L.) Cout.	Geophyte	5	Capsule	None	Seed	Coriaceous	Endosperm	ca. 0.5	III–X

$p < 0.05$), *S. heldreichii* ($\rho = 0.173$, $p < 0.05$) and their combination ($\rho = +0.204$, $p < 0.05$). 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 ($\rho = +0.184$, $p < 0.05$), 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 be tween 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 mediumto 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 habitats 100s of m away where mother plants were absent from the standing vegetation (Figs. 3 & 4).

Figure 1. Propagules described in Table 2. (A) Cypsela with vilano of Inula Crithmoides; (B) seed of Spergularia heldreichii; (C) seeds of Arthrocnemum macrostachyum; (D) utricules and accesory fruits of Atriplex halimus; (E) accesory fruits of Halimione portulacoides; (F) seeds of Salicornia ramosissima; (G) utricules and accesory fruits of Salsola vermiculata; (H) spikelets of Limoniastrum monopetalum; (L) Spikelets of Limonium algarvense; (M)

spikelets Limonium ferulaceum; (N) fruiting calyces of Limonium narbonense; (O) florets Puccinelia convoluta; (P) spikelets of Spartina maritima; (Q) seed of Polygonum equisetiforme; and (R) seeds of Cistanche phelipaea

3.6 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 freshwater 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.

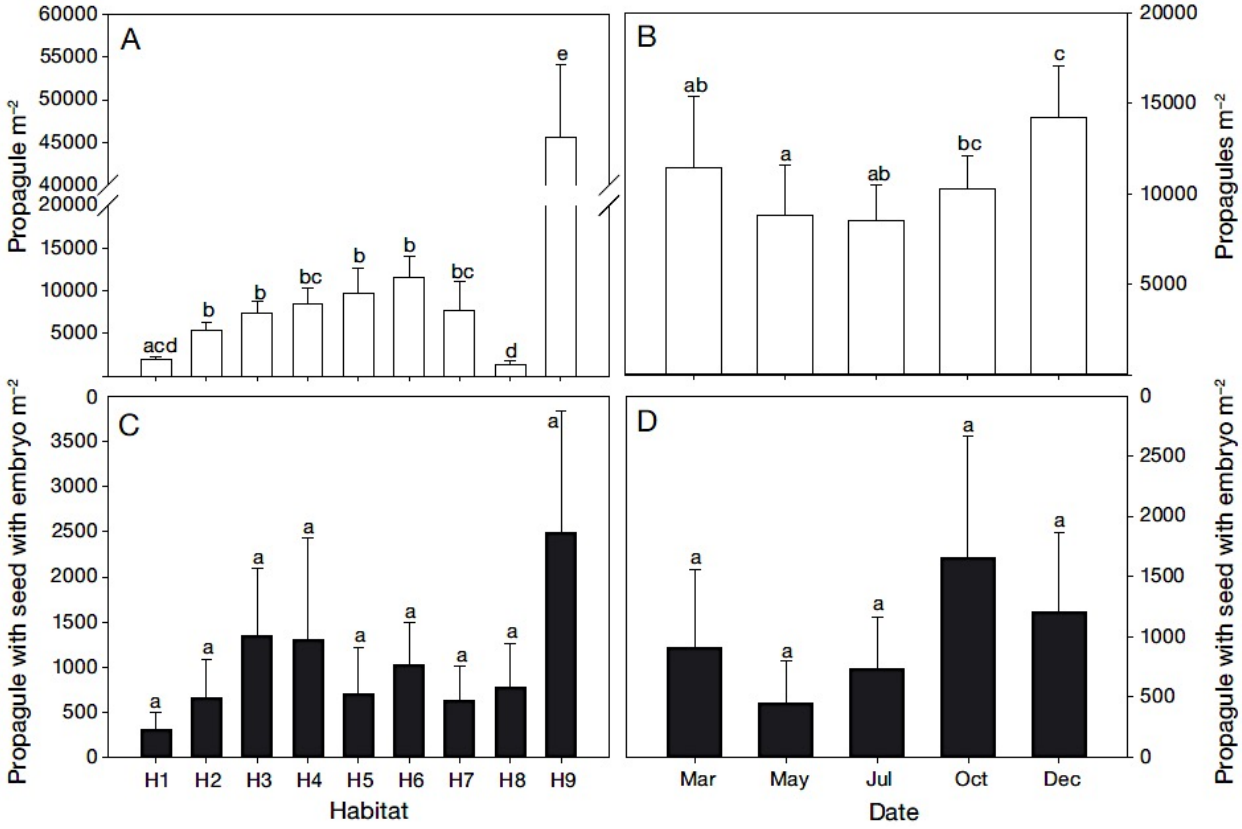


Fig. 2. Density of (A,B) total propagules and (D,C) those that contain seeds with embryon of halophytes in the sediments at 9 salt marsh hábitats (H1-H9) along the intertidal gradient (A,C) and in 5 months (March, May, July, October and December) (B,D) at Ría Formosa (southwest Iberian Peninsula). Different lowercase letters indicate significant differences between hábitats 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 *Haliminone portulacoides* and *Sarcocornia fruticosa*; H5: *Inula crithmoides* formations; H6: high marshes of *Arthrocnemum macrostachyum*; H7: hight marshes of *Limoniastrum monopetalum*; H8: Sandy sediments coloniced by *Salsola vermiculata* L.; H9: ecotone between marshes and coastal dunes. Value are mean ±SE (n = 45 samples habitat^{-1} H2-H9 and n = 60 in H1; n = 84 samples date-^{1})

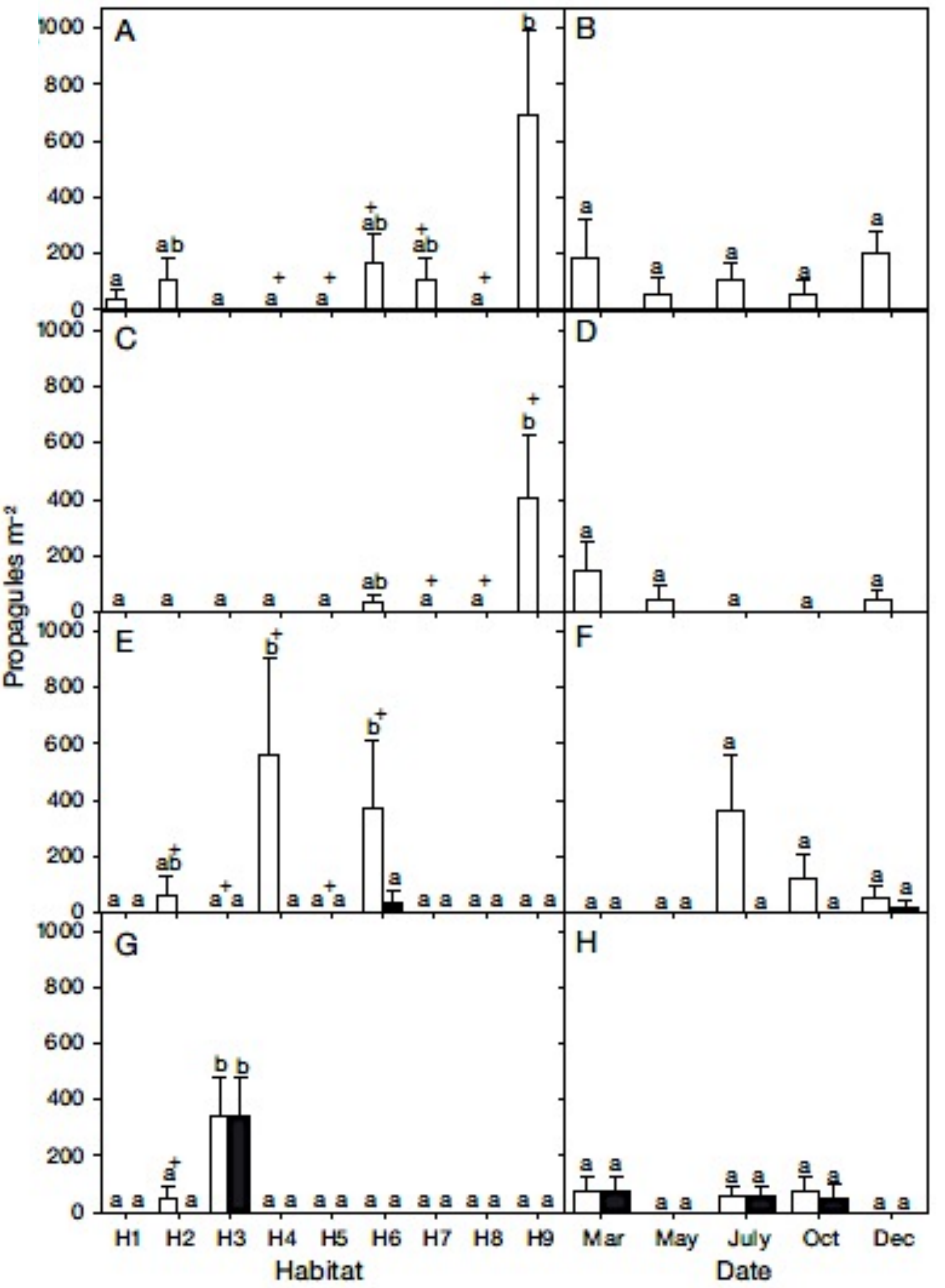


Fig.3. Density of propagules (White bars: total; black bars: containing seed with embryo) of (A,B) *Limonium ferulaceum* plus *L. diffusum*, (C,D) *Salsola vermiculata*, (E,F) *Halimione portulacoides* and (G,H) *Salicornia ramosissima* in the sediments at 9 salt marsh hábitats (H1-H9) along the intertidal 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 hábitat. Different lowercase letters indicate significant differences between hábitats or dates (Mann-Whitney U-test, $p < 0.05$). See Fig. 2 for habitat legend. Values are mean ± SE (n = 45 samples habitat^{-1} H2-H9 and n = 60 in H1; n= 84 samples date^{-1})

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 crithmo ides (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^{-1}) 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 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 x 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 área 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 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 behind 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*

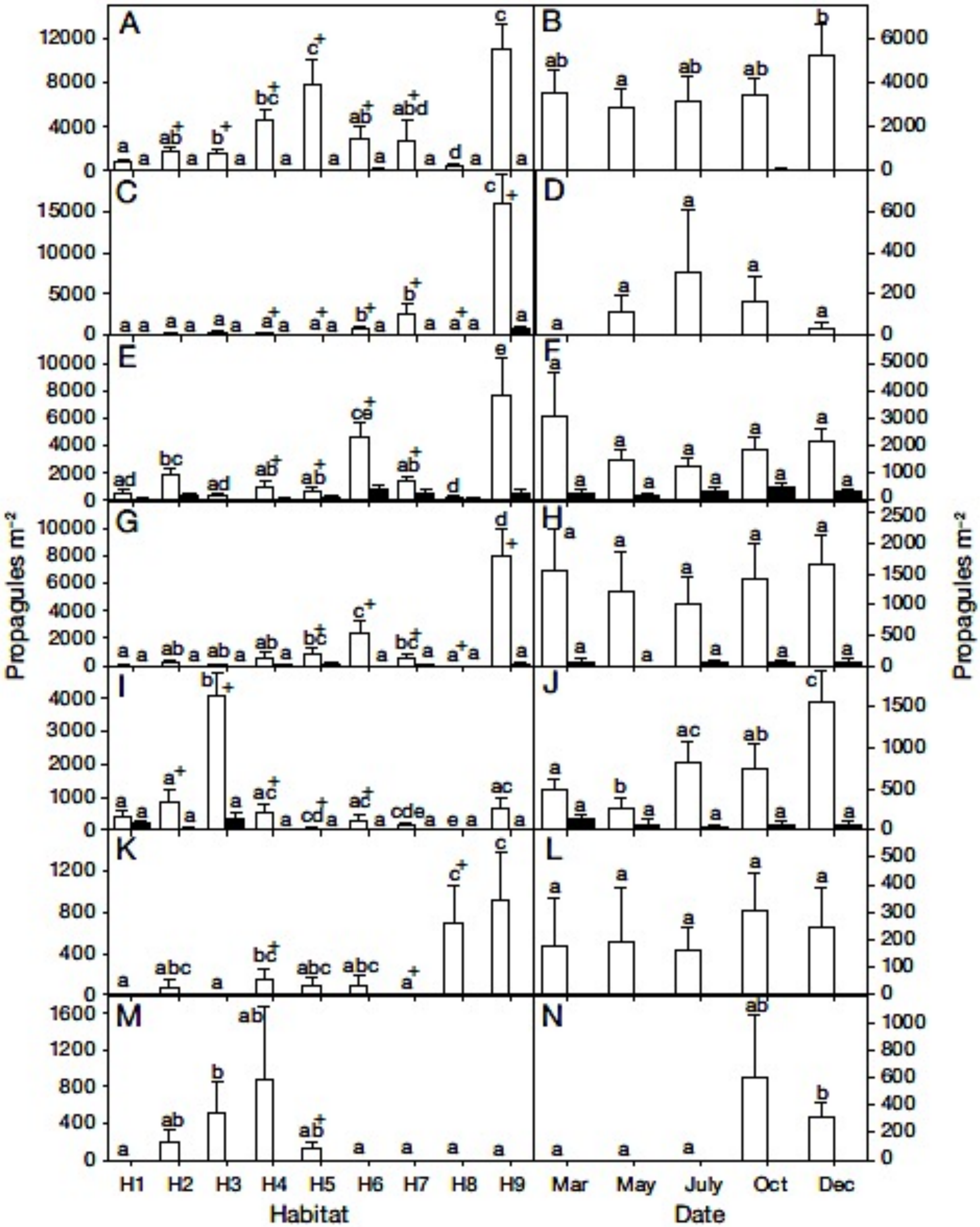


Fig. 4. Density of propagules (White bars: total; black bars: containing seed with embryo) of (A,B) *Suaeda sp.*, (C,D) *Limoniastrum monopetalum*, (E,F) *Artrhrocnemum macrostachyum*, (G,H) *Limonium algarvense,* (I,J) *Sarcocornia sp*., (K.L) *Spergularia sp*., and (M,N) *Cistanche phelypaea* in the sediments at 9 salt marsh hábitats (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 hábitat. Different lowercase letters indicate significant differences between hábitats or dates (Mann-Whitney U-test, $p < 0.05$). See Fig. 2 for habitat legend. Values are mean ± SE (n = 45 samples habitat^{-1} H2-H9 and n = 60 in H1; n= 84 samples date^{-1})

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 hábitats 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^{-1} 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).

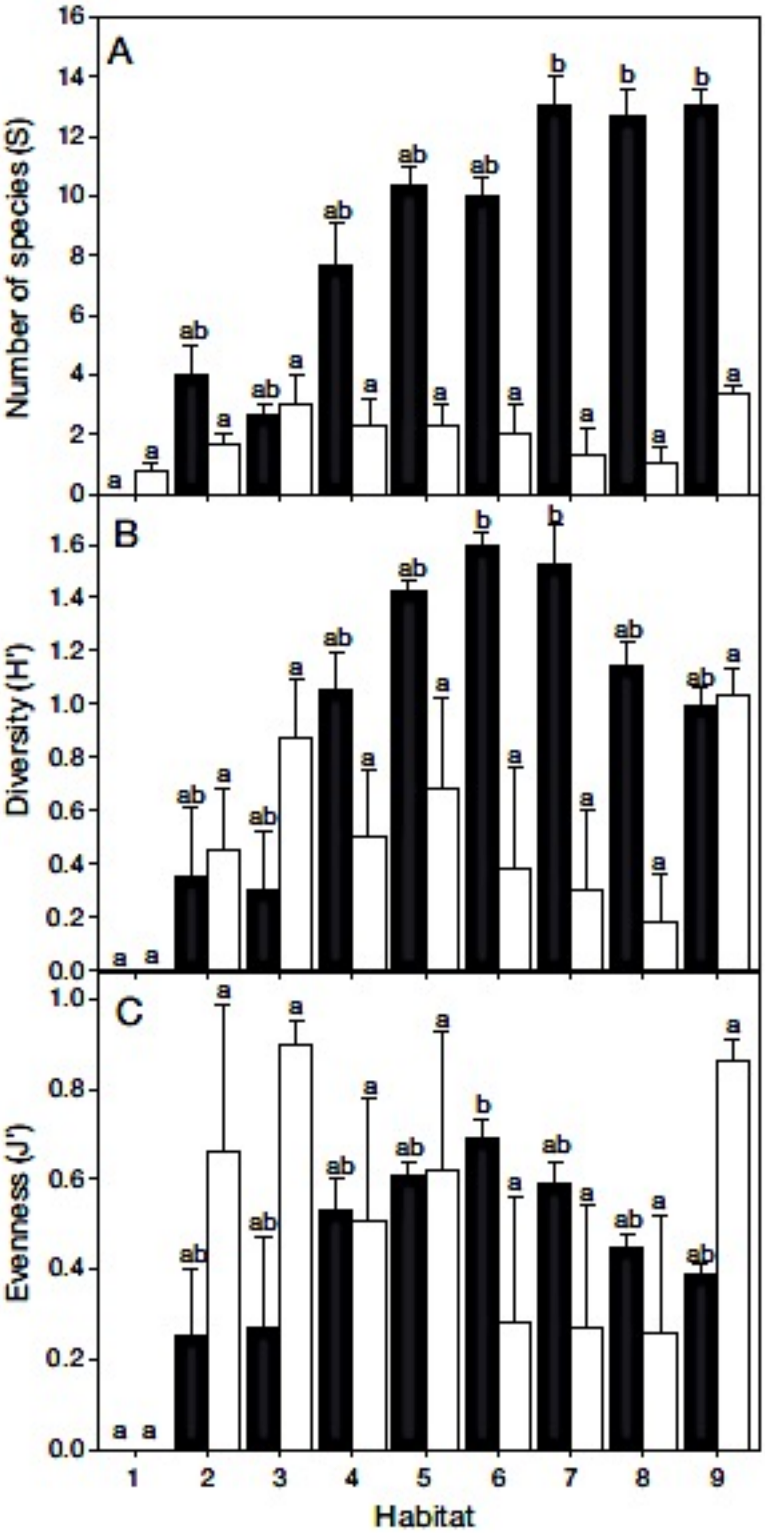


Fig. 5. (A) Species richness, (B) biological diversity and (C) evenness of the halophylic vegetation (black columns) and its seed bank (White columns) at 9 salt marsh hábitats (H1-H9) along the intertidal gradient at Ría Formosa (southwest Iberian Peninsula). Different lowercase letters indicate significant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

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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4. Artículo 3

Seed bank dynamics of the annual halophyte *Salicornia ramosissima*: towards a sustainable exploitation of its wild population

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Seed bank dynamics of the annual halophyte *Salicornia ramosissima*: towards a sustainable exploitation of its wild population

4.1 Abstract

Halophytes are able to survive in the high salted areas of the world, and have been recognized as ources of bioactive metabolites. There is a need to design sustainable strategies for the use of wild populations of halophytic species in order to avoid irrational gathering. Seed banks are essential for resilience and regeneration in salty ecosystems. We sampled annual seed production, aerial and soil seed banks and seed dynamics for a year in four wild populations of the annual halophyte *Salicornia ramosissima* growing in saltpans, in order to develop sustainable management practices for the use of its populations. The seed production of *S. ramosissima* depended mainly on plant density rather than on the number of seeds produced by each individual plant. In three of the four study populations, most of the annual seed production was exported out of the saltpans (79%) and only between 14 and 20% was accumulated in the initial aerial and soil seed banks. These initial seed banks were highly depleted during the year until the next fruiting period, when they accumulated less than 1% of the annual seed production (from 19 to 15,302 seed m^{-2}). *Salicornia ramosissima* established a persistent soil seed bank in two of the four locations. Annual seed production would be key for the preservation of those *S. ramosissima* populations that do not establish persistent soil seed banks. In view of our results, each population of *S. ramosissima* should be studied independently to design population-specific management plans.

4.2 Key words

Annual seed production, Odiel marshes, salinity, saltpan, seed dispersal, soil seed bank.

4.3 Introduction

Salt-tolerant plants (halophytes) have been traditionally used as healthy and functional foods and medicines for human and animals (Marinoni et al. 2019; ElNaker et al. 2020). More recently, various halophyte species have been recognized as sources of bioactive primary and secondary metabolites of pharmacological, cosmetic, biofuel and nutraceutical interest (Debez et al. 2017, Petropoulos et al. 2018; Faustino et al. 2019).

Salt stress negatively affects soil fertility, causing land degradation. In fact, drought and soil salinity are the main factors responsible for crop yield reduction in the present scenario of climate change and sea level rise (Alae-Carew et al. 2020). Nevertheless, halophytes are able to survive in the high salted areas of the world inhabited by an ever-growing number of people (Fita et al. 2015). In this context, halophyte cultivation would seem to be cheaper than other comercial crops, and can yield industrial value, remediate nutrient-rich effluents from aquaculture and rehabilitate lands through soil desalination (Custodio et al. 2017; Nikalje et al. 2018). Besides 'saline agriculture', wild populations of halophytes can be exploited as sources of 'wild food', but very few studies have analyzed the key ecological aspects of halophytes in the development of sustainable management practices (Godfray et al. 2010). Most studies have focused on analyzing the traditional uses of wild halophyte populations by local people and their bioactive properties (Pereira et al. 2020). Since people have become aware of the potential of some halophytes as part of a healthy diet, their natural growth sites are now threatened in some locations (Nae-Kyu and Lee 2012). Thus, there is a need to design sustainable strategies for the use of wild populations of halophytic species in order to avoid irrational gathering and genetic erosion threats.

Halophytes colonize saline environments, such as salt marshes, where soil salinity determines plant distribution, as high salinity conditions affect seed survival and germination (Ungar 1987). Salinity reduces germination and stimulates dormancy in halophyte seeds (Pujol et al. 2000). Some plant species survive in unfavorable environmental conditions in saline environments by building on persistent seed banks (< 1 year). Other plant species have transient seed banks (> 1 year) as the result of high and rapid germination and/or because their seeds quickly die in the soil (Walck et al. 2007; Parsons 2012). The reserves of mature viable seeds in the form of seed banks can be repositories of genetic information located on the plants (aerial seed bank) and in the soil (soil seed bank) (Roberts 1981). Germination from halophyte seed banks occurs mainly during germination windows, when soil salinity decreases after rainfalls (Gul and Weber 2001; Noe and Zedler 2001). So, seed banks are essential for resilience, secondary succession and regeneration in salty ecosystems (Honnay et al. 2007). Seed banks may be especially important for the long-term preservation of annual halophytes that may be absent in the aboveground vegetation and present in the seed bank waiting for favorable conditions (Egan and Ungar 2000).

Amaranthaceae (formerly known as Chenopodiaceae) is one of the most represented family of halophytes, with many species that are well known as plants of pharmacological and nutraceutical interest (Lefevre and Riviere 2020). Specifically, the species of the genus *Salicornia* L. have a broad geographical distribution and are among the most frequent halophytes with pharmacological and culinary uses. An array of functional nutrients has been detected in *Salicornia* species, justifying its usage as a 'sea vegetable' (Patel 2016). *Salicornia ramosissima* J. Woods is an annual extremophile halophyte that presents apparently leafless, succulent and articulated stems (Valdés 1987). *Salicornia ramosissima* colonizes European and North African salt marshes occurring in a whole range of salt marsh habitats, such as saltpans, where it germinates during winter–spring and dies off during autumn–winter (Davy et al. 2001). *Salicornia ramosissima* plants are fit for human consumption (Lima et al. 2020) and are useful for the ecological restoration of saline areas (Santos et al. 2017). The germination of this annual halophyte decreases at salinities higher than 0.2 M NaCl and is rapidly activated after salinity release (Rubio-Casal et al. 2003 ; Muñoz-Rodríguez et al. 2017). Although seed banks play a significant role in the optimum development of *S. ramosissima* populations (Egan and Ungar

1999; Silva et al. 2007; van Regteren et al. 2019), no study has analyzed in detail the aerial and soil seed banks of any *Salicornia* species and their seasonal dynamics.

Our main goal was to analyze *S. ramosissima* seed bank size and dynamics to help to design sustainable exploitation strategies of wild populations. We hypothesized that annual seed production of *S. ramosissima* would be very high, contributing to the establishment of large and permanent soil seed banks in each population. To test this hypothesis, we sampled annual seed production, aerial and soil seed banks and seed dynamics for a year in different populations of *S. ramosissima* colonizing saltpans. Based on our results, we propose sustainable management practices for the use of wild populations of *Salicornia* as biomass sources.

4.4 Materials and methods

4.4.1 Study area

The present study was carried out in tidal marshes in the Odiel Marshes Natural Park (37º 12′ 32.3′′ N, 6º 58′ 01.5′′ W, Gulf of Cádiz, Southwest Iberian Peninsula) (Online Resource 1—Fig. S1). The mean tidal range is 2.10 m, and the mean spring tidal range is 2.97 m in the Odiel Marshes. The semidiurnal tides can lead to hypersalinity in some mature marshes (Castellanos et al. 1994). The Odiel Marshes are subjected to a Mediterranean climate with Atlantic influence. Mean temperatures are + 17–24 ºC, and annual precipitation is 250–850 mm with 75–85 days of rain during the autumn and winter months, and a 4–5-month dry period from approximately June–September (AEMET 2018), when potential evapotranspiration exceeds precipitation.

Saltpans are frequent in salt marshes in a Mediterranean climate. They are depressed areas with high salinity concentrations located at high salt marsh zones, where hypersalinity excludes colonization by most plant species (Rubio-Casal et al., 2001). Saltpans are the main habitat of *S. ramosissima* together with low marshes (Rubio-Casal et al. 2001; Contreras-Cruzado et al. 2017). We studied four populations of *Salicornia ramosissima* colonizing four different saltpans: Population 1 (P1) was located at a pond of an abandoned salt mine with no drainage to tidal channels, where *S. ramosissima* colonized the periphery of a central unvegetated mud flat, just below the halophilous scrubby vegetation growing on the pond's containment dams (37º 15′ 41.6′′N, 6º 58′ 35.54′′ W); Population 2 (P2) (37º 13′ 39.63′′ N, 6º 57′ 46.52′′ W) and Population 3 (P3) (37º 13′ 34.74′′ N, 6º 57′ 50.54′′W) were colonizing the sediments surrounding the bare central areas of saltpans inundated during spring high tides. The halophilous scrub vegetation occupied higher elevations than *S. ramosissima* in the saltpans; Population 4 (P4) was located along drainage channels in a saltpan with dense scrub vegetation (37º 13′ 33.09′′ N, 6º 57′ 52.87′′ W) (Fig. S1). Three zones were differentiated in each study location to analyze the dispersal of *S. ramosissima* seeds within saltpans: (1) unvegetated sediments at the lowest elevations of the saltpan; (2) sediments colonized by monospecific communities of *S. ramosissima*; and (3) sediments colonized by halophilous scrubs, such as *Arthrocnemum machrostachyum* (Moric.) C. Koch, *Limoniastrum monopetalum* (L.) Boiss. and *Atriplex halimus* L., at the highest elevations of the saltpans (Online Resource 2, Fig. S2). Plants species were identified following Valde´s et al. (1987).

4.4.2 Sedimentary environment

We collected three sediment samples in each of the three zones of the four study saltpans in October 2017. Sediment samples were collected randomly using stainless steel cores of 50 mm diameter and 50 mm height. Samples were placed in hermetically closed polyethylene bags and stored at -5 ºC until analysis in the laboratory. Sediment electrical conductivity was used as a measure of soil salinity (Richards 1974). From each sample, a mix of 10 ml of sediment and distilled water (1:1,v:v) was homogenized, and the conductivity measured in the unfiltered supernatant with a conductivity meter (Crison Instruments 5064, Hach Lange, Barcelona, Spain). Sediment pH was measured in the same unfiltered supernatant used for conductivity, using a pH meter (Crison 25, Hach Lange, Barcelona, Spain) (Nieva et al. 2001). Sediment water content was gravimetrically determined using samples of 30 g of sediment (Contreras-Cruzado et al. 2017). Sediment organic matter content was determined by the loss-on-ignition method. Organic matter content was calculated as the proportion of weight lost as compared to the weight of the dry sample before incineration (Gavlak et al. 2005).

4.4.3 Annual seed production

Live plant density at the end of the flowering period of *S. ramosissima*, when the vast majority of the plants had ripened fruits, was recorded by counting the total number of live plants in 10 randomly chosen plots (20 x 20 cm) inside the *S. ramosissima* zone in each population in October 2017 for P1, P2 and P3, and in November 2017 for delayed P4; withered plants from the previous flowering period were not counted. Seed production per plant was recorded for 30 randomly collected plants in each population. The production of seeds per plant was calculated in the laboratory using two methods. For small plants (with less than 50 seeds), we counted all their seeds under optical microscope. For large plants (with more than 50 seeds), we separated all the branches from the principal axis and weighed them. Then, three randomly chosen branches were weighed individually and their total number of seeds counted under optical microscope. The seed production per plant was calculated as the product between the quotient of seeds per weighed unit and the total weight of the braches for each plant. Annual seed production per plot was calculated as the product between plant density and seed production per plant. Finally, we calculated mean annual seed production per square meter in each population.

4.4.4 Aerial seed bank

The aerial seed bank or storage of seeds on the plants after seed dispersal, was studied at two moments. The initial aerial seed bank included seeds retained by the current year plants, and it was recorded just after the current seed dispersal in November 2017 for P1, P2 and P3, and in December 2017 for P4. The remnant aerial seed bank included seeds retained by plants just before the seed dispersal of the next flowering period, and it was recorded in October 2018 for each population (Fig. 1). In both cases, we calculated the number of seeds retained per plant following the same methodology reported previously for annual seed production. The initial aerial seed bank was calculated using the density of live plants, and the remnant aerial seed bank was obtained using the density of withered plants from the previous flowering period that still remained in the population (Fig. 1).

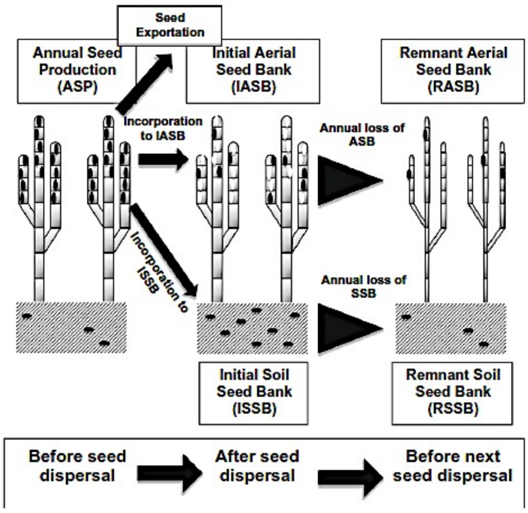

Figure 1. Schematic representation of the dynamics of seed production in *Salicornia ramosissima* and of aerial and soil seed banks from the moment before seed dispersal to the same moment of the next flowering period.

4.4.5 Soil seed bank

The soil seed bank was studied at two moments: the initial soil seed bank, just after the current seed dispersal (recorded in October 2017 for P1, P2 and P3, and in November 2017 for P4), and the remnant soil seed bank, just before the seed dispersal of the next flowering period (recorded in October 2018 for each population) (Fig. 1). In each sampling, we randomly took 10 sediment samples per zone (unvegetated, *S. ramosissima* and scrub zones) at the four study saltpans using stainless steel cores of 50 mm diameter and 50 mm height. Sediment samples were placed in polyethylene bags, hermetically sealed and transported to the laboratory for analysis. In the laboratory, the sediment samples were frozen until analyzed. Dry sediment samples were sieved through a 0.4 mm-light sieve to separate the seeds from sediments, and the material retained on the sieve was examined under a magnifying glass (Polo-Ávila et al. 2019).

4.4.6 Seed dynamics

We calculated the percentage of the annual seed production incorporated into initial aerial and soil seed banks at the *S. ramosissima* zone, into the soil seed bank in adjacent vegetation zones and dispersed out of the study saltpans. The annual loss of aerial and soil seed banks was calculated as a percentage of seeds in the initial seed banks not present in the remnant seed banks (Fig. 1).

4.4.7 Data analysis

The data were analyzed using Statistica 8.0 (StatSoft INC., USA). Deviations from the arithmetic means were calculated as standard error (SE). Significant differences were considered when $p < 0.05$. Data series or their transformations (using log (x + 1), 1/ (x + 1) or $\sqrt{x}$ functions) were tested for homogeneity of variance and normality with the Levene test and the Kolmogorov–Smirnov test, respectively. The data series were compared between populations or vegetation zones using an one-way ANOVA and Tukey's test as post hoc analysis. When transformed data series did not show a normal distribution or homogeneity of variance, they were analyzed using the Kruskal–Wallis (H) and Mann–Whitney U tests with population or vegetation zones as grouping factors. The nonparametric Spearman's correlation coefficient (q) was used to analyze the relationships between initial and remnant aerial seed banks, annual seed production and density of plants.

4.5 Results

4.5.1 Sedimentary environment

P1 showed the highest electrical conductivity, at 70% higher than that recorded for P4 ($F_{3,11} = 33.415$, $p < 0.0001$) (Fig. 2a). In contrast, sediment pH reached its highest value in P4, and its lowest value in P2 and P3 ($H_{3,36} = 25.04$, $p < 0.0001$; U test, $p < 0.05$) (Fig. 2b). There were no significant differences for sediment water or organic matter content between populations (water content: $F_{3,11} = 1.258$, $p = 0.352$; Organic matter content: $H_{3,12} = 5.760$, $p = 0.124$) (Fig. 2c, d).

4.5.2 Annual seed production

Live plant densities ranged from 330 ± 47 plants m^{-2} for P3 to 3610 ± 1018 for P4 ($H_{3,40} = 18.46$, $p < 0.0005$; U test, $p < 0.05$) (Fig. 3a). Seed production per live plant did not show significant interpopulation differences ($H_{3,120} = 3.72$, $p = 0.293$) (Fig. 3b). Annual seed production ranged from 48,743 ± 6937 seed m^{-2} for P3 to 2,179,383 ± 614,577 seed m^{-2} for P4 ($H_{3,40} = 24.68$, p\ 0.0001; U test, $p < 0.05$) (Fig. 3c).

4.5.3 Aerial seed bank

The number of seeds retained by each *Salicornia* plant just after the current dispersal was lower for P2 (7 ± 3 seed $plant^{-1}$) than for the other three populations (c. 50 seed $plant^{-1}$) ($H_{3,120} = 24.56$, $p < 0.0001$; U test, $p < 0.05$) (Fig. 3b). The initial aerial seed bank varied between 2848 ± 700 seed m^{-2} for P2 and 298,645 ± 84,217 seed m^{-2} for P4 ($H_{3,40} = 30.07$, $p < 0.0001$; U test, $p < 0.05$) (Fig. 3c), increasing together with the density of the live plants ($\rho = + 0.738$, $p < 0.0001$, $n = 40$) and their annual seed production ($\rho = + 0.678$, $p < 0.0001$, $n = 40$).

The density of remnant dead plants from the previous year was the lowest for P2 and P3 (c. 400 plant m^{-2}) and the highest for P4 (1570 ± 533 plant m^{-2}) ($H_{3,40} = 8.54$, $p < 0.05$; U test, $p < 0.05$)

(Fig. 3a). Seeds retained by these dead plants were lower than 0.1 seed plant^{-1} for P1 and P3, and reached its maximum (9.75 ± 3.23 seed plant^{-1}) in P4 ($H_{3,120} = 39.50$, $p < 0.0001$; U test, $p < 0.05$) (Fig. 3b). The remnant aerial seed bank changed from c. 25 seed m^{-2} for P1 and P3 to 15,302 ± 5,196 seed m^{-2} for P4 ($H_{3,40} = 29.14$, $p < 0.0001$; U test, $p < 0.05$) (Fig. 3c), increasing together with the density of the remnant plants ($\rho = + 0.639$, $p < 0.0001$, $n = 40$).

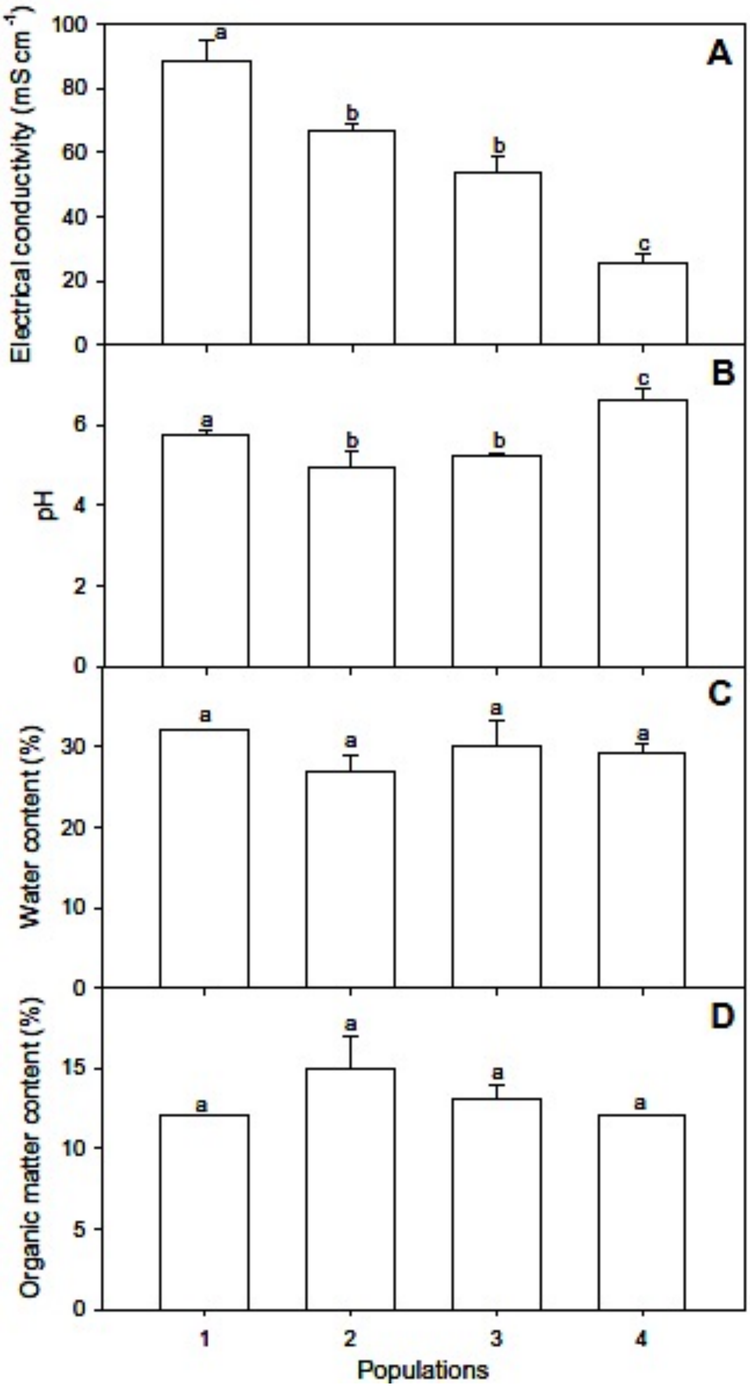

Figure 2. Sediment electrical conductivity (**a**), pH (**b**), water content (**c**) and organic matter content (**d**) in four *Salicornia ramosissima* populations colonizing saltpans in the Odiel Marshes (Southwest Iberian Peninsula). Different letters indicate significant differences between populations (Tukey's test or Mann–Whitney test, $p<0.05$). The data are mean ± SE (n = 3)

4.5.4 Soil seed bank

The initial soil seed bank in *S. ramosissima* zones was similar for each population ($H_{3,120} = 1.98$, $p = 0.577$), whereas the remnant soil seed bank tended to be higher at P1 and P2 (c. 350 seed m^{-2}) than at P3 and P4, where no seed was recorded (H3,120 = 7.53, p = 0.057) (Fig. 4). In the four study saltpans, the initial soil seed bank was the highest in the zone colonized by *S. ramosissima*, but while it was significantly different to that reached in the two other zones for P2 and P4, it was similar to that in the adjacent soils colonized by halophylous scrubs for P1 and P3 (P1, $H_{2,30} = 11.21$, $p < 0.005$; P2, $H_{2,30} = 20.16$, $p < 0.0001$; P3, $H_{2,30} = 8.75$, $p < 0.05$; P4, $H_{2,30} = 19.72$, $p < 0.0001$) (Fig. 4). In contrast, the remnant soil seed bank was similar in each vegetation zone at every study saltpan, always lower than 500 seed m^{-2} (Kruskal–Wallis test, $p > 0.05$) (Fig. 4).

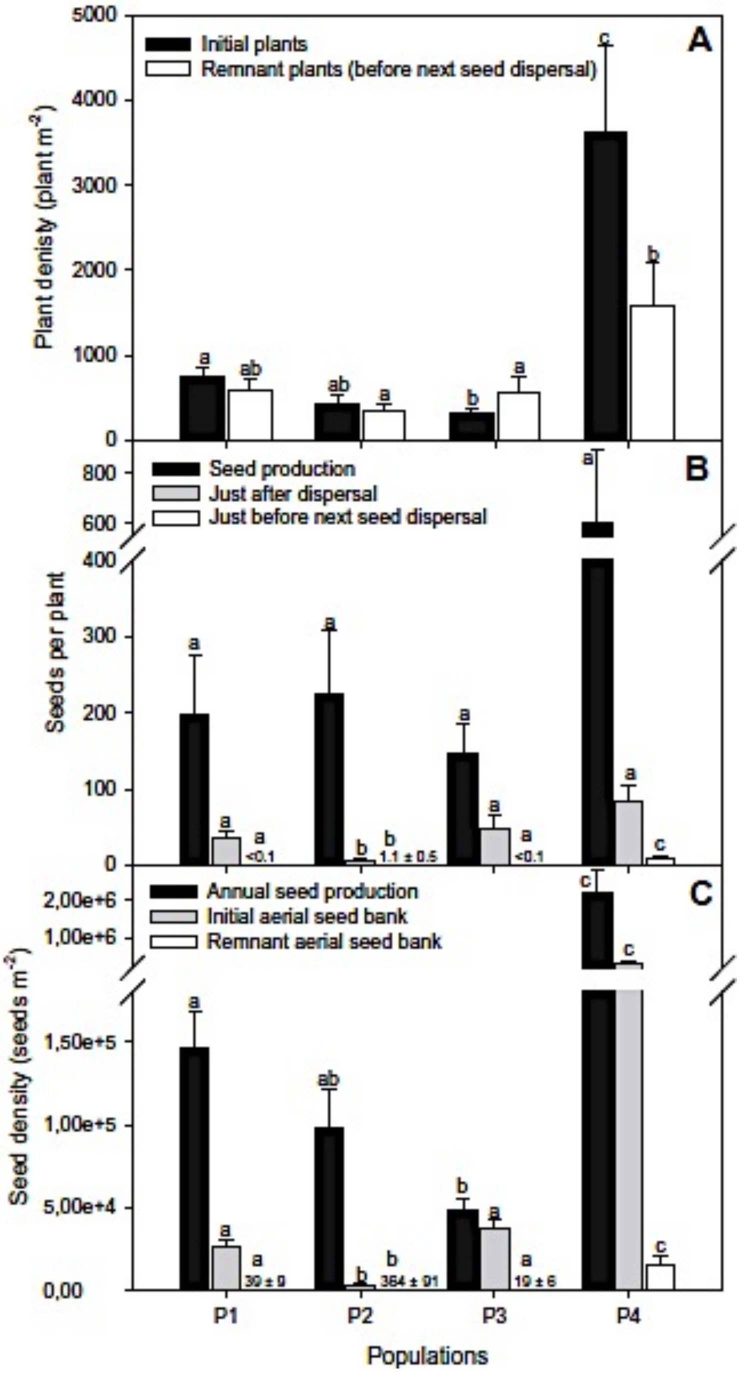

Figure 3. Plant density (**a**), seed production and number of seeds per plant (**b**) and annual seed production and aerial seed banks just after seed dispersal and just before next seed dispersal for four populations of *Salicornia ramosissima* (P1–4) colonizing saltpans in the Odiel Marshes (Southwest Iberian Peninsula). Different letters indicate significant differences between populations for the same trait (Mann–Whitney test, $p<0.05$). The data are mean ± SE (n = 10)

4.5.5 Seed dynamics

The percentage of the annual seed production retained in the initial aerial seed bank ranged from 2.91% for P2 to 75.93% for P3. The seeds accumulated in the initial soil seed bank varied from 0.36% for P4 to 17.10% for P2. Less than 1.00% of the annual seed production was dispersed from *S. ramosissima* zones to other zones in the study saltpans for each population. Predation and dispersal out of the saltpans were between 18.60% (P3) and 85.93% (P4) of the anual seed production (Fig. 5).

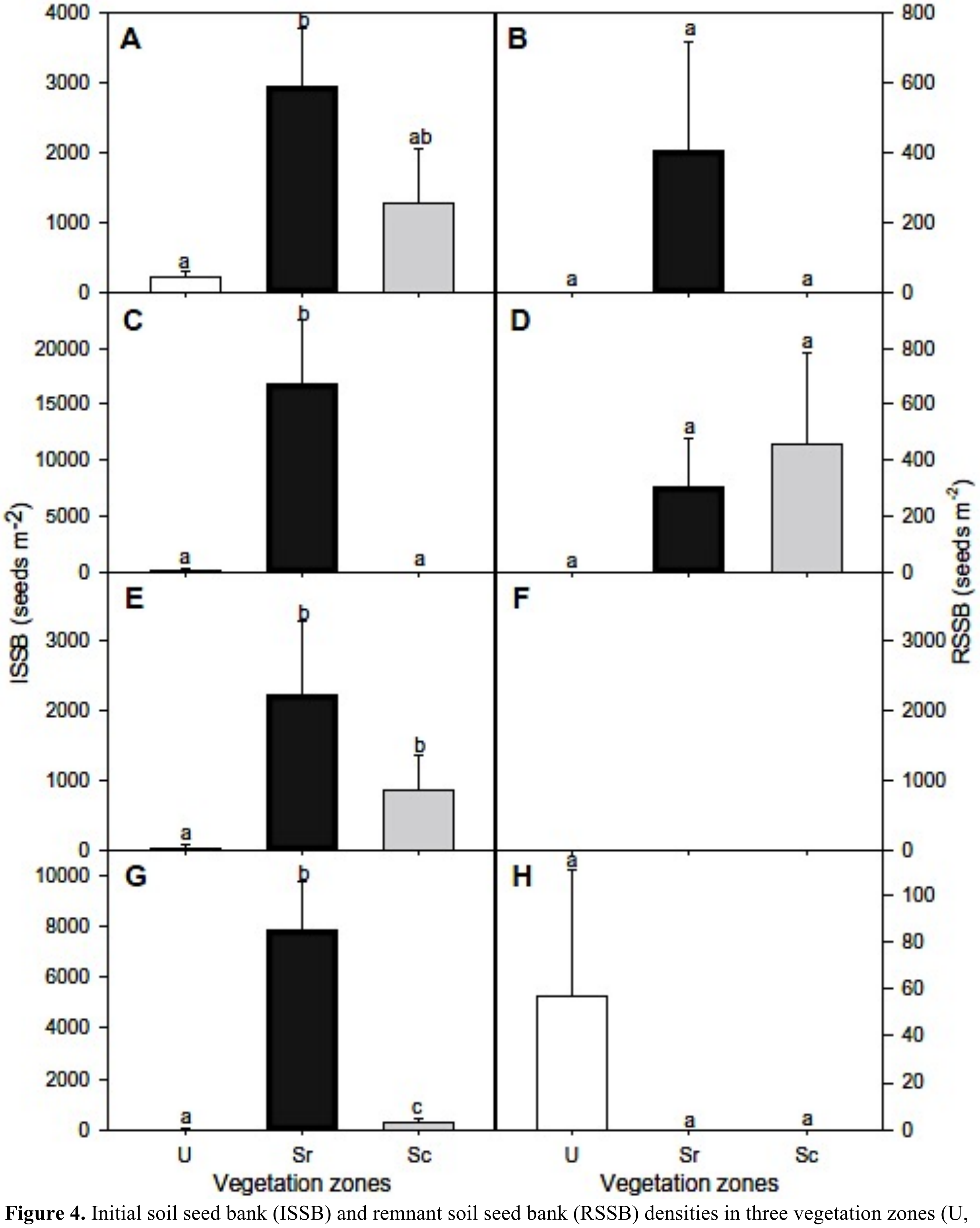

Figure 4. Initial soil seed bank (ISSB) and remnant soil seed bank (RSSB) densities in three vegetation zones (U, unvegetated (White columns); Sr, *S.ramosissima* (black columns); Sc, scrubs (grey columns)) for four *Salicornia ramosissima* populations (P1 (**a**, **b**), P2 (**c**, **d**), P3 (**e**, **f**), P4 (**g**, **h**)) growing in saltpans in the Odiel Marshes (Southwest Iberian Peninsula). Different letters indicate significant differences between vegetation zones (Mann–Whitney test, $p<0.05$). The data are mean ± SE (n = 10).

The percentage of the initial aerial seed bank predated and dispersed from the plants throughout the year ranged from c. 71.84% for P2 and P4 to c. 99.87% for P1 and P3 ($H_{3,40} = 19.8$, p<

0.0001; U test, $p < 0.05$). Thus, the percentage of seeds retained in the remnant aerial seed bank was always lower than 0.10% of the annual seed production, ranging from 19 to 15,302,374 seeds m^{-2} (Figs. 3 and 5). The percentage of seeds depleted from the initial soil seed bank during the year ranged from c. 92.55% for P1 and P2 to 100% for P3 and P4 ($H_{3,40} = 8.2$, $p = 0.043$; U test, $p < 0.05$; U test, $p < 0.05$). These percentages corresponded to less than 0.35% of the annual seed production for each population, varying from 0 to 407 seeds m^{-2} (Figs. 4 and 5).

4.6 Discussion

Our results show that the seed production of the annual halophyte *S. ramosissima* depends mainly on plant density rather than on the number of seeds produced by each individual plant. In three of the four study populations, most of the annual seed production was exported out of the saltpans (> 79%), and only between 14 and 20% was accumulated in the initial aerial and soil seed banks. These initial seed banks were highly depleted during the year until the next fruiting period, when they accumulated less than 1% of the annual seed production (from 19 to 15,302 seed m^{-2}). In fact, *S. ramosissima* established a persistent soil seed bank in only two of the four populations. In this context, annual seed production would be key for the preservation of those *S. ramosissima* populations that do not establish persistent soil seed banks.

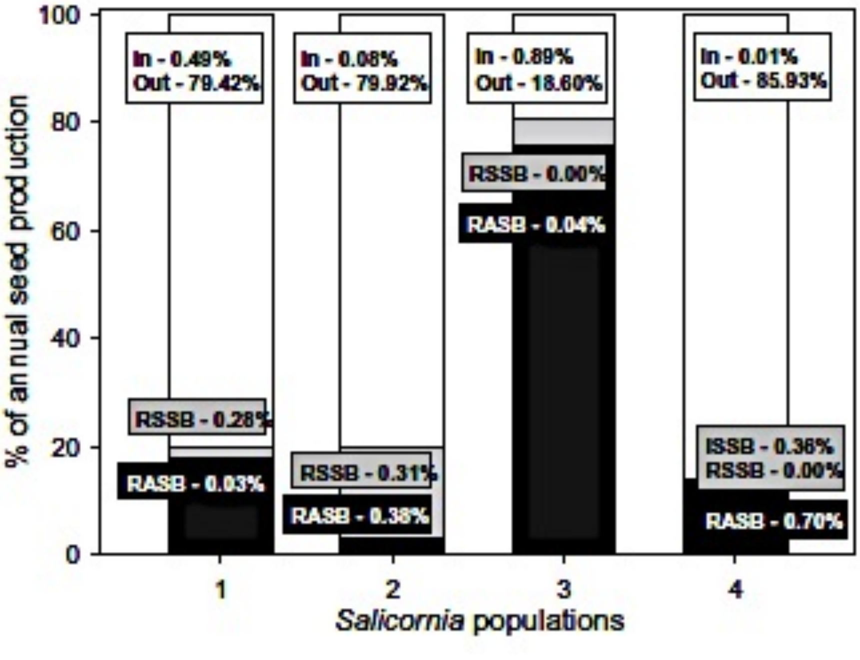

Figure 5. Percentages of annual seed production accumulated in the initial aerial seed bank (black), remnant aerial seed bank (RASB), initial subterranean seed bank (ISSB; gray), remnant soil seed bank (RSSB), total exported (white), exported within saltpans (In) and predated and exported out of saltpans (Out) from four populations of Salicornia ramosissima (P1–4) in the Odiel Marshes (Southwest Iberian Peninsula). The data are mean (n = 10).

We recorded high annual seed production (> 48,000 seed m^{-2}) for *S. ramosissima* growing in the harsh environmental conditions of saltpans marked by high sediment salinities (16–63 ppt). High salinity induces *S. ramosissima* seed dormancy without affecting seed viability (Rubio-Casal et al. 2003; Muñoz-Rodríguez et al. 2017). According to Davy et al. (2001), plant density varies greatly among *Salicornia* populations, regulated by a combination of density-dependent seed production and density-independent seedling mortality due to high levels of morphological phenotypic plasticity. In our study, annual seed production increased with the density of individual plants regardless of the seed production per individual plant, which was similar for each population. In fact, the highest seed production per individual plant (604 seeds plant^{-1}) was obtained for the population with the highest plant densities (3610 mature plants m^{-2}), rending

2,179,383 ± 614,577 seeds m^{-2}. This was probably due to the low plant densities recorded in our populations in relation to other studies that have reported close to 30,000 mature plants m^{-2} (Davy et al. 2001).

The seed dynamic changed markedly between *Salicornia* populations. For example, between 14.1 and 80.5% of the annual seed production (19,603–306,539 seeds m^{-2}) was stored in aerial and soil seed banks. The initial aerial seed bank was larger than the initial soil seed bank in three of the four study populations, accumulating more than 2,800 seeds m^{-2} in each population. Aerial seed banks help seed dispersal over time, and may protect seeds from being predated in the soil (Santini and Martorell 2013) and from unfavorable soil conditions such as high salinities (El-Keblawy and Bhatt 2015). Between 71.8 and 99.8% of the initial aerial seed bank may have been dispersed or predated during the first year, whereas the initial soil seed bank was totally depleted in two of the four study populations. The remnant aerial seed bank also increased together with the density of plants, storing between 19 and 15,302 seeds m^{-2} in different populations. Thus, between 19.5 and 85.9% of annual seed production was predated and exported out of *Salicornia* populations just after seed dispersal. *Salicornia ramosissima* shows a short-distance dispersal strategy since its seeds have hooker hairs that help them to anchor to sediments and vegetation (Polo-Ávila et al. 2019). *Salicornia* seeds disperse mostly on the soil surface since they float for less than one day (Huiskes et al. 1995). Genetic analyses have shown a strong tendency to inbreeding as a result of a lack of seed immigration from outside Salicornia populations (Davy et al. 2001). These previous observations together with our results, which show that less than 0.9% of the annual seed production was dispersed from *Salicornia* populations to adjacent zones in each study saltpan, suggest that predation would be more important than seed exportation in study populations.

Polo-Ávila et al. (2019) stated that *S. ramosissima* establishes persistent seed banks, but we recorded that the soil seed bank was drastically reduced, even totally depleted, during the first year after seed dispersal. This result is in line with Jefferies et al. (1981), who recorded the depletion of the seed bank of *Salicornia europaea* L. in the middle of the first summer following dispersal. The diminution of *S. ramosissima* seeds from its soil seed bank may be due to its high and fast germination during favorable conditions (Parsons 2012), and to seed predation recorded in different *Salicornia* species (Davy et al. 2001). Tessier et al. (2000) recorded the absence of a persistent seed bank for the annual species *Suaeda maritima* (L.) Dumort. due to very high germination during low salinity periods. The transitory condition of the soil seed bank for some *S. ramosissima* populations recorded in our study is in accordance with previous studies of different *Salicornia* species (Philipupillai and Ungar 1984; Thompson et al. 1997; Wolters and Bakker 2002; Rubio-Casal et al. 2003).

4.7 Conslusions

In view of our results, each wild population of *S. ramosissima* should be studied independently to design population-specific management plans for sustainable exploitation. For example, the establishment of a large persistent soil seed bank on some populations enables the collection of high percentages of adult plants (c. 70–95% of the total population; 233–415 plants m^{-2}), ensuring the formation of an initial soil seed bank with double the number of seeds than the recorded number of mature plants. In contrast, plant collection should be limited to 9% of the mature plants (c. 308 plants m^{-2}) in other populations to achieve the same goal. In any case, more than 230 plants m^{-2} could be extracted from each study population, ensuring the formation of an initial soil seed bank with double the number of seeds than the recorded number of mature plants (Online Resource 3, Table S1). In addition, reintroduction experiments by sowing *S. ramosissima* seeds should be carried out in parallel with studies on the sustainable harvesting of *S. ramosissima* in order to create new populations and reinforce currently existing populations, as reported for *S. europaea* (Nae-Kyu and Lee 2012).

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5. Artículo 4

Population dynamic of the annual halophyte *Salicornia ramosissima* in salt pans: towards a sustainable exploitation of its wild populations

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Population dynamic of the annual halophyte *Salicornia ramosissima* in salt pans: towards a sustainable exploitation of its wild populations

5.1 Abstract

Halophyte species growing under stressful conditions, such as the annual species of the *Salicornia* genus, have been recognized as a source of metabolites of pharmacological and nutraceutical interest. Therefore, planning the extraction of individual plants from wild populations in a sustainable way is especially important in the case of annual species. We studied the environmental matrix and population dynamic of four *Salicornia ramosissima* populations growing at two elevations in salt pans under a Mediterranean climate. In elevated areas, *S. ramosissima* populations presented maximum plant densities of between 628–6288 plants m^{-2} that remained almost constant until fruiting. In contrast, populations in depressed zones presented five-times greater soil-seed-bank densities and maximum plant densities than populations in elevated zones. In this context, populations in depressed zones lost c. 60% of their maximum plant densities from the end of spring and through summer. In whatever way the environmental matrix seemed to control the population dynamic of *S. ramosissima* in depressed zones, the effects of a stressful environment would interact with plant densities. In this sense, we recorded the density-dependent mortality for the densest population (max. 51,558 plants m^{-2}). Our results are useful for planning a sustainable harvesting of natural populations of *S. ramosissim*a.

4.2 Key words

Air temperature; inland marshes; Odiel Marshes; plant density; saline agricultura; salinity; salt marsh.

5.3 Introduction

Halophytes are salt-tolerant plants that colonize saline environments such as salt marshes where they play a key ecological role, for instance, promoting ecosystem structuring and ecological succession [1]. In addition, halophyte species growing under stressful conditions have been recognized as a source of metabolites of pharmacological and nutraceutical interest [2 –4]. In this context, the exploitation of wild populations of halophytes offers a great opportunity as a form of saline agriculture [5], yet halophyte preservation is threatened by several anthropogenic impacts, including unsustainable exploitation of their wild populations [6]. Therefore, sustainable management plans need to be designed for the exploitation of wild populations of halophytic species in order to avoid irrational gathering.

Most halophytes are perennials, but some halophyte species are annuals [7]. Planning the extraction of individual plants from wild populations in a sustainable way is especially important in the case of annual halophyte species, since their populations are generated each year from pre existing seed banks [8]. To our knowledge, no previous study has analyzed the population dynamic of annual halophyte species to develop sustainable management practices.

The Amaranthaceae family (formerly known as Chenopodiaceae) presents many species that are well known as plants of pharmacological and nutraceutical interest [9]. One of its most popular genera is Salicornia L. that includes annual succulent halophyte plants whose stems and seeds are used in fresh salads, bread or tea [10, 11], and as a source of nutraceutical and pharmacological compounds [12 –15]. Specifically, *Salicornia ramosissima* J. Woods colonizes European and North African salt marshes, occurring in a wide range of habitats [16], including salt pans, since their seeds are able to germinate at hypersalinity [17, 18]. The seed production of *S. ramosissima* mainly depends on plant density rather than on the number of seeds produced by each individual plant and its soil seed bank, which is drastically reduced, even totally depleted, during the first year after seed dispersal [8], as has been reported for *Salicornia europa*ea L. [19].

The survival of annual *Salicornia* populations has been investigated in coastal and inland salt marshes [19–21]. Plant density for *Salicornia* species varies greatly among populations and is regulated by a combination of density-dependent seed production and density-independent mortality [19, 22–24]. Thus, *Salicornia* plants grow in dense populations that could reach densities greater than 100,000 m^{-2}, but most studies carried out in North European marshes indicated that although density-dependent intraspecific competition can reduce the growth of *Salicornia* plants, high plant densities did not increase mortality [16]. Only [25] found density dependent seedling mortality in populations of *S. ramosissima* growing under an *Arthrocnemum macrostachyum* (Moric.) Moris canopy in salt pans under a Mediterranean climate, where environmental conditions were less stressful than in open salt pans and where mortality was not related to plant density. In this context, soil salinity, waterlogging, the mechanical impact of tidal action, burial by sediments and predation are among the main environmental factors determining the death of *Salicornia* plants [16, 20, 26–29]. In the stressful environment of salt marshes, *Salicornia* populations exhibit high levels of phenotypic plasticity, genetically fixed differences in growth phenology [30] and local adaptation to their habitats [23 ,31 ,32]. Thus, individual populations of Salicornia may be highly sensitive to elevation variations in salt marshes, as populations from lower areas are more tolerant of prolonged submergence and waterlogging [16], while populations in upper areas are exposed to a higher risk of drought [20]. In this sense, *Salicornia* europaea have been described as less tolerant to drought than the perennial *Sarcocornia fruticosa* (L.) A.J. Scott. [33]. Although in some populations of *S. ramosissima* outbreeding by wind pollination is not completely avoided due to the existence of protandry [34], *Salicornia* species are seen as selfing species whose populations can be considered homozygous lineages with low genetic diversity [16 ,35 ,36].

Regardless of the considerable ecological and socioeconomic interest in *Salicornia* species, the vast majority of studies on their population dynamic were carried out exclusively on the North

Atlantic coasts of Europe and North America despite the observed variability that exists between species, populations and habitats [16]. In the present work, we studied the environmental matrix and population dynamic of four *S. ramosissima* populations, from seedling establishment to fructification, growing at two elevations in salt pans under a Mediterranean climate. Our first hypothesis was that plant survival within a population would be density-independent and, secondly, we hypothesized that mortality would be governed by environmental factors affecting populations differently depending on their elevation. Our results are useful for planning sustainable exploitation strategies of wild populations of *Salicornia* under the stressful conditions of a Mediterranean climate, especially in the present climate-change scenario.

5.4 Results

5.4.1 Meteorological and Sedimentary Environment

The mean air temperature was 19.4 ºC, the mean maximum temperature was 24.1 ºC and the mean minimum was 14.7 ºC from December 2019 to September 2020. The rainfall was 345.0 mm during the study period (Figure 1).

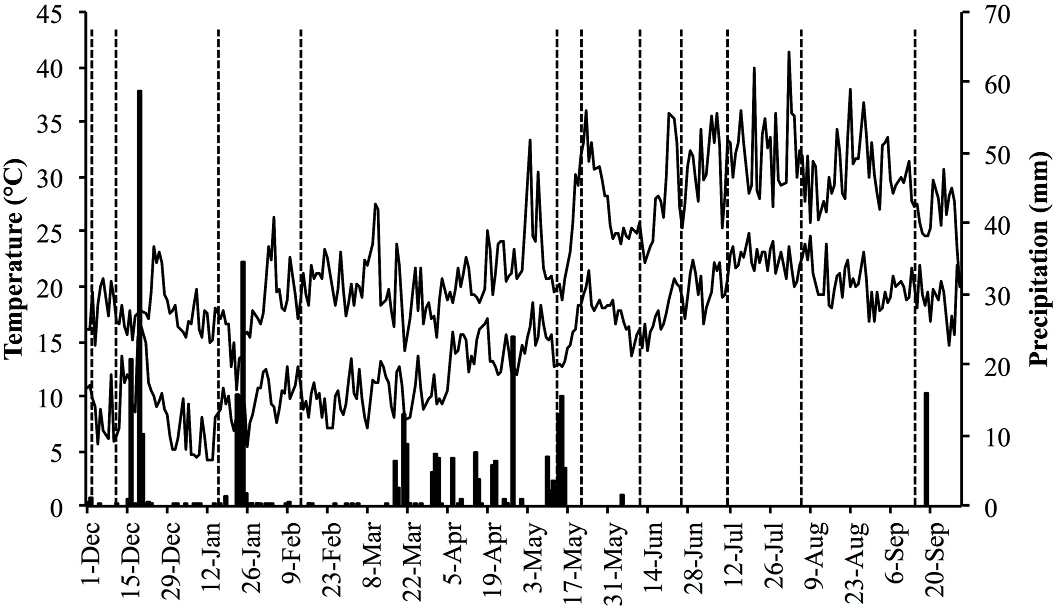

Figure 1. Daily maximum and minimum air temperature (ºC) (lines) and precipitation (mm) (columns) from December 2019 to September 2020 in the Odiel Marshes. Vertical dashed lines indicated sampling dates.

The sediment pH varied between 6.5 and 7.0 in different *S. ramosissima* populations, being 3% higher in the elevated zones than in the depressed zones (Tables 1 and 2). The sediment pH reached its maximum in January and its minimum in June–July for all populations (Figure 2 A, Table 2).

Table 1. Sediment pH and electrical conductivity (EC) (mS cm^{-1}) and seed-bank density (seed m^{-2}) for four populations of *Salicornia ramosissima*, and in elevated and depressed zones. Different letters indicate significant differences among populations (Kruskal-Wallis test, $p < 0.05$) or between elevated and depresed zones (Mann-Whitney test, $p < 0.05$. Data are mean ± SE.

	1	2	3	4	Elevated Zones	Depressed Zones
Soil seed bank (seed m^{-2})	23,474 ^{a} ± 9595 (n = 15)	7671 ^{a} ± 1767 (n = 15)	27,162 ^{ab} ± 6356 (n = 15)	124,620 ^{b} ± 30,825 (n = 15)	15,572 ^{A} ± 5013 (n = 30)	75,891 ^{B} ± 17,916 (n = 30)
Soil pH	6.8 ^{a} ± 0.1 (n = 81)	6.5 ^{c} ± 0.1 (n = 81)	7.0 ^{b} ± 0.1 (n = 81)	6.7 ^{a} ± 0.0 (n = 81)	6.6 ^{A} ± 0.0 (n = 162)	6.8 ^{B} ± 0.0 (n = 162)
Soil conductivity (mS cm^{-1})	54.7 ^{a} ± 5.0 (n = 81)	35.9 ^{b} ± 3.3 (n = 81)	45.5 ^{ab} ± 3.9 (n = 81)	32.7 ^{b} ± 2.0 (n = 81)	45.3 ^{A} ± 3.1 (n = 162)	39.1 ^{B} ± 2.2 (n = 162)

The mean annual sediment EC varied between 33±2 and 55±5 mS cm^{-1}, with no significant differences between physiographic zones (Tables 1 and 2). The lowest values of sediment EC were recorded during winter and the highest values in summer for all populations (Figure 2B, Table 2).

Table 2. Kruskal–Wallis test and Mann–Whitney U-test comparing between four populations of *Salicornia ramosissima*, two salt-pan elevations and nine sampling points for sedimentary and plant variables.

	Between Populations	**Between Elevations**	**Between Sampling Dates**
Sediment pH	$H_{(3,324)} = 49.75, p < 0.0001$	$U = 2886.0, p < 0.001$	P1: $H_{(8,81)} = 54.92, p < 0.0001$; P2: $H_{(8,81)} = 56.39, p < 0.0001$; P3: $H_{(8,81)} = 64.90, p < 0.0001$; P4: $H_{(8,81)} = 61.35, p < 0.0001$
Sediment electrical conductivity (mS cm^{-1})	$H_{(3,324)} = 10.91, p = 0.012$	$U = 3847.5, p = 0.562$	P1: $H_{(8,81)} = 71.09, p < 0.0001$; P2: $H_{(8,81)} = 65.24, p < 0.0001$; P3: $H_{(8,81)} = 65.07, p < 0.0001$; P4: $H_{(8,81)} = 63.63, p < 0.0001$
Seed-bank density (seeds m^{-2})	$H_{(3,60)} = 24.22, p <= 0.0001$	$U = 160.0, p < 0.0001$	-
Maximum plant density (plants m^{-2})	$H_{(3,53)} = 29.53, p < 0.0001$	$U = 115.0, p < 0.0001$	-
Density of surviving plants at the end of the study (plants m^{-2})	$H_{(3,53)} = 34.22, p = 0.0000$	$U = 281.5, p = 0.246$	-
Density of fruiting plants (plants m^{-2})	$H_{(3,53)} = 34.22, p < 0.0001$	$U = 273.5, p = 0.190$	-
Proportion of fruiting plants in relation to plant density at the end of the study	$H_{(3,53)} = 1.51, p = 0.6802$	$U = 186.0, p = 0.524$	-
Proportion of fruiting plants in relation to maximum plant density	$H_{(3,53)} = 18.20, p = 0.0004$	$U = 174.0, p = 0.002$	-
Proportion of surviving plants in relation to maximum plant density	$H_{(3,53)} = 19.28, p = 0.0002$	$U = 145.0, p = 0.0002$	-
Plant height (cm)	$H_{(3,53)} = 15.83, p = 0.0012$	$U = 200.5, p = 0.622$	-

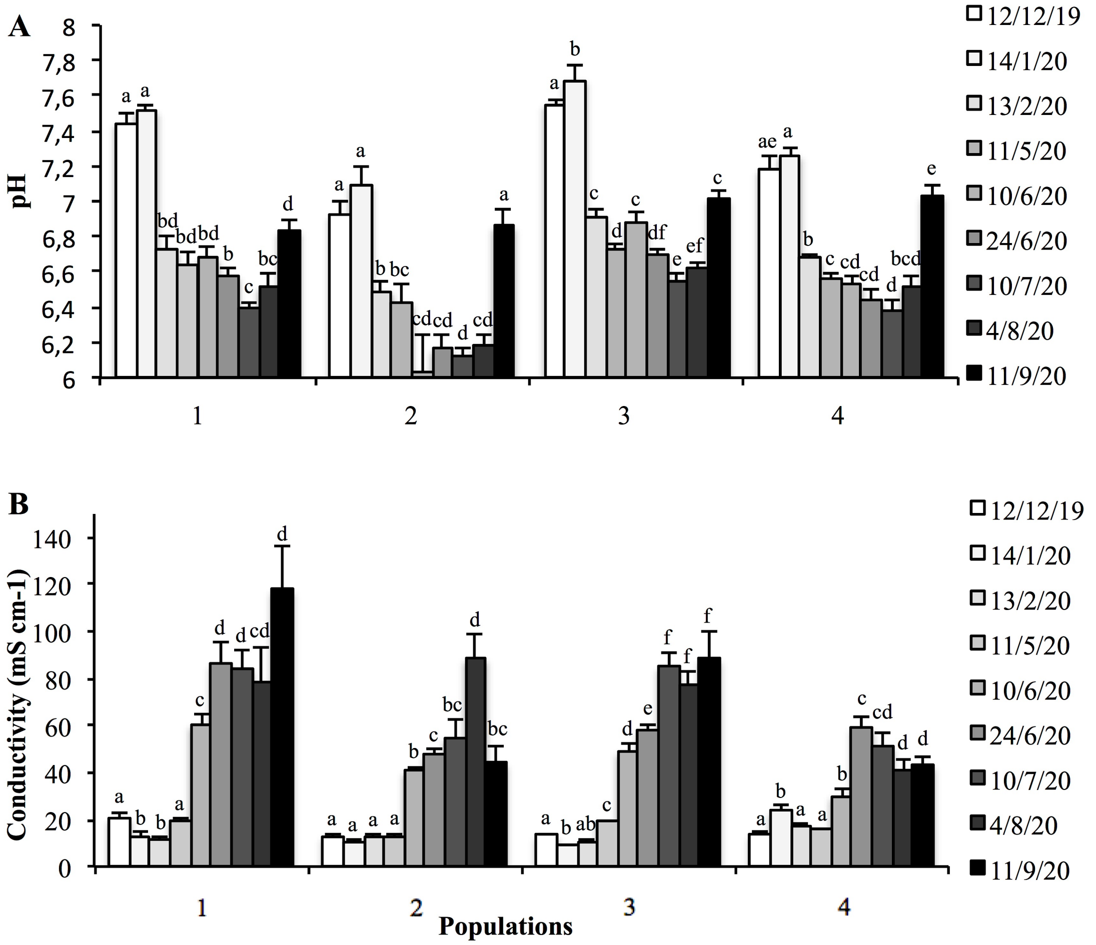

Figure 2. Sediment pH (**A**) and electrical conductivity (mS cm^{-1}) (**B**) for four *Salicornia ramosissima* populations from December 2019 to September 2020. Different letters indicate significant differences between dates for the same population (Mann–Whitney test, $p < 0.05$). Values are mean ±SE.

5.4.2 Soil Seed Bank and Population Dynamic of *Salicornia ramosissima*

The seed-bank density of *S. ramosissima* varied between 7,671 ± 1,767 seeds m^{-2} for P2 and 124,620 ± 30,825 seeds m^{-2} for P4, being 79% higher in depressed zones than in elevated zones (Tables 2 and 3).

Table 3. Population parameters for four populations of *Salicornia ramosissima*, and for populations in elevated and depressed zones. Different letters indicate significant differences among populations (Kruskall–Wallis test, $p < 0.05$) or between physiographic zones (Mann Whitney test, $p < 0.05$). Values are mean ± SE.

	1	**2**	**3**	**4**	**Elevated Zones**	**Depressed Zones**
Mean sampling date (in ordinal number) when maximum plant density was reached	$3.75^{a} \pm 0.60$ ($n = 12$)	$3.55^{a} \pm 0.88$ ($n = 11$)	$3.13^{a} \pm 0.26$ ($n = 15$)	$2.73^{a} \pm 0.23$ ($n = 15$)	$3.65^{A} \pm 0.51$ ($n = 23$)	$2.93^{A} \pm 0.17$ ($n = 30$)
Mean maximum plant density reached (plant m^{-2})	$2777.0^{ab} \pm 565.9$ ($n = 12$)	$1714.8^{b} \pm 255.6$ ($n = 11$)	$3521.1^{a} \pm 541.23$ ($n = 15$)	$18{,}946.6^{c} \pm 3682.6$ ($n = 15$)	$2269.00^{a} \pm 332.47$ ($n = 23$)	$11{,}233.82^{b} \pm 2322.81$ ($n = 30$)
Mean plant density at last sampling date (plant m^{-2})	$1362.3^{a} \pm 335.9$ ($n = 12$)	$1028.9^{a} \pm 175.25$ ($n = 11$)	$419.2^{b} \pm 169.9$ ($n = 15$)	$4149.8^{c} \pm 377.3$ ($n = 15$)	$1202.84^{A} \pm 193.30$ ($n = 23$)	$2284.49^{A} \pm 401.62$ ($n = 30$)
Mean proportion of survival plants (plant density at last sampling date respect to maximum plant density reached)	$0.46^{ac} \pm 0.09$ ($n = 12$)	$0.64^{a} \pm 0.10$ ($n = 11$)	$0.11^{b} \pm 0.04$ ($n = 15$)	$0.34^{c} \pm 0.06$ ($n = 15$)	$0.54^{A} \pm 0.07$ ($n = 23$)	$0.23^{B} \pm 0.04$ ($n = 30$)
Mean density of blooming plants at last sampling date (plant m^{-2})	$1152.7^{a} \pm 335.9$ ($n = 12$)	$857.4^{a} \pm 153.4$ ($n = 11$)	$335.3^{b} \pm 135.4$ ($n = 15$)	$4066.0^{c} \pm 372.6$ ($n = 15$)	$1011.48^{A} \pm 188.50$ ($n = 23$)	$2200.66^{A} \pm 397.38$ ($n = 30$)
Mean proportion of blooming plants at last sampling date respect to plant density at last sampling date	$0.83^{a} \pm 0.12$ ($n = 10$)	$0.87^{a} \pm 0.07$ ($n = 10$)	$0.96^{a} \pm 0.04$ ($n = 6$)	$0.98^{a} \pm 0.01$ ($n = 15$)	$0.85^{A} \pm 0.07$ ($n = 20$)	$0.97^{A} \pm 0.02$ ($n = 21$)
Mean proportion of booming plants at last sampling date respect to maximum plant density reached	$0.39^{a} \pm 0.10$ ($n = 12$)	$0.54^{a} \pm 0.10$ ($n = 11$)	$0.08^{b} \pm 0.03$ ($n = 15$)	$0.33^{a} \pm 0.06$ ($n = 15$)	$0.47^{A} \pm 0.07$ ($n = 23$)	$0.21^{B} \pm 0.04$ ($n = 30$)
Mean height of plants at last sampling date	$6.40^{a} \pm 0.94$ ($n = 10$)	$12.92^{b} \pm 0.96$ ($n = 10$)	$11.79^{abc} \pm 3.17$ ($n = 7$)	$8.17^{c} \pm 0.52$ ($n = 15$)	$9.66^{A} \pm 0.99$ ($n = 20$)	$9.32^{A} \pm 1.08$ ($n = 22$)

The plant density increased from December to February for every *S. ramosissima* population, then the populations in elevated zones maintained constant values until flowering (Figure 3A), whereas plant density abruptly decreased (c. 60%) to the end of the study for the populations in depressed zones (Figure 3B). The daily variation in plant density in each sampling ring in proportion to the maximum plant density increased together with daily rainfall only in the elevated zones (Figure 4A, Table 4). In contrast, the daily variation in plant density decreased when the maximum air temperature and sediment EC increased in the depressed zones (Figure 4B, C, Table 4).

Table 4. Spearman correlation coefficient (ρ) and probability value (p) for correlations between daily variation in plant density in proportion to maximum density (plants m^{-2}), and daily variation in different environmental variables for four populations of *Salicornia ramosissima* and for populations in elevated and depressed zones. Significant values are marked in bold ($p < 0.05$).

Maximum Plant Density vs.		**1**	**2**	**3**	**4**	**Elevated Zones**	**Depressed Zones**
Plant density at last sampling date	r	**0.823**	0.579	0.404	**0.542**	**0.801**	**0.811**
	p	**0.0010**	0.0618	0.1349	**0.0368**	**0.0000**	**0.0000**
Proportion of survival plants	r	0.133	−0.389	0.221	**−0.897**	−0.092	0.223
	p	0.6803	0.2372	0.4293	**0.0000**	0.6748	0.2371
Density of blooming plants at last sampling date	r	**0.685**	0.600	**0.521**	**0.543**	**0.693**	**0.829**
	p	**0.0140**	0.0512	**0.0463**	**0.0364**	**0.0002**	**0.0000**
Proportion of blooming plants respect to maximum plant density reached	r	0.032	−0.350	0.397	**−0.908**	−0.126	0.335
	p	0.9211	0.2913	0.1421	**0.0000**	0.5664	0.0703
Mean height of plants at last sampling date	r	−0.518	0.222	0.019	0.098	−0.348	0.022
	p	0.1255	0.5370	0.9683	0.7274	0.1332	0.9242

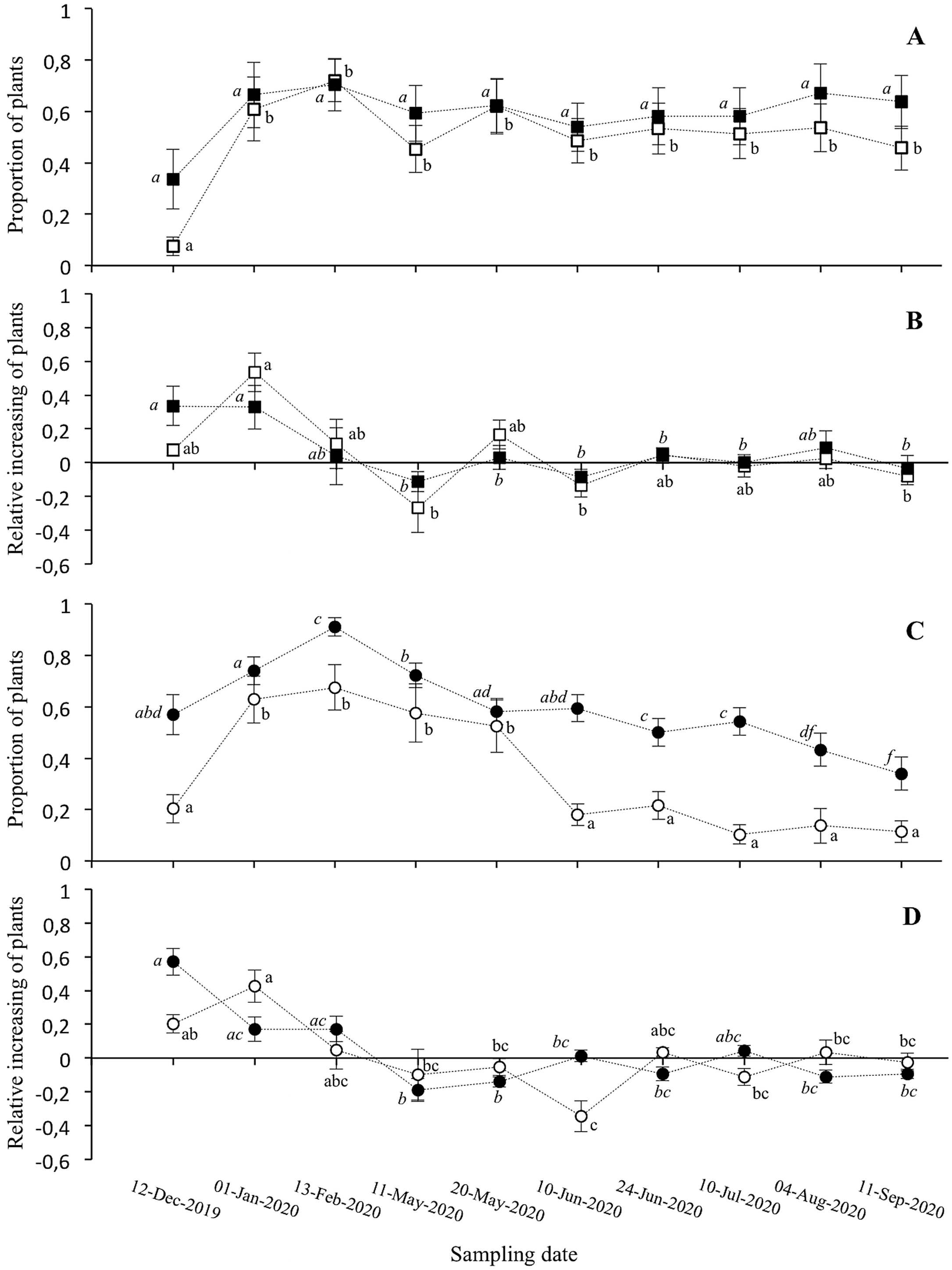

Figure 3. Density of plants (**A**,**C**) and increasing plant density (**B**,**D**) in relation to the maximum density reached for four populations of Salicornia ramosissima colonizing (**A**) elevated zones (Population 1, white squares; Population 2, black squares) and (**C**) depressed zones (Population 3, white circles; Population 4, black circles) from December 2019 to September 2020. Values are mean ± SE.

The maximum density of *S. ramosissima* plants varied between 1715 ± 256 plants m^{-2} for P2 and 18,947 ± 3683 plants m^{-2} for P4, being five-times greater in depressed zones than in elevated zones (Tables 2 and 3). Thus, the maximum plant density increased together with soil-seed-bank density ($\rho = 0.760$, $p = 0.0041$, $n = 12$), representing between 14 ± 3% for P1 and 31 ± 17% for P2 of the soil-seed-bank density, with no significant differences among populations (Kruskal–Wallis test: $H(3,12) = 2.08$, $p = 0.5566$).

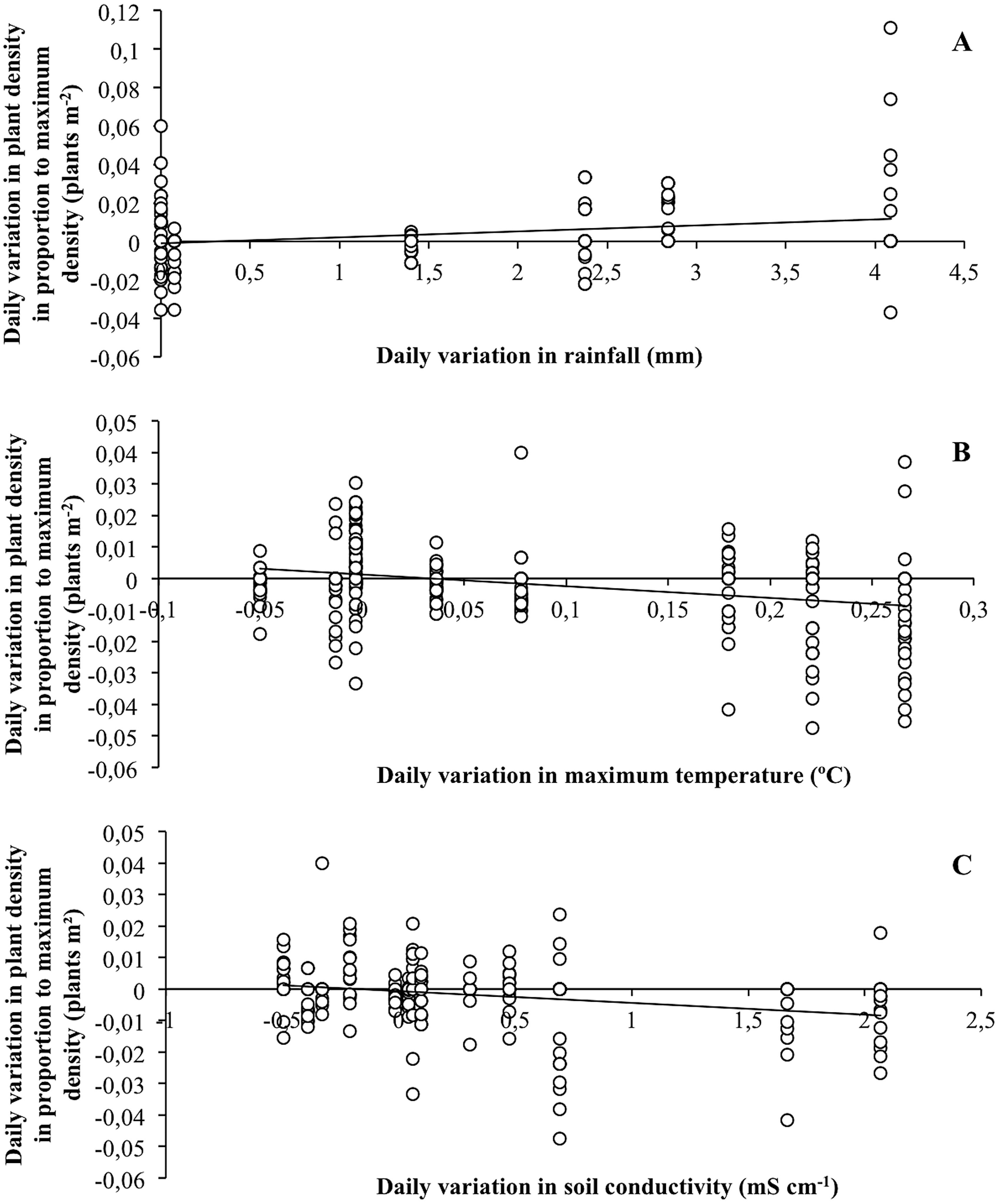

Figure 4. Relations between daily variation in plant density (plant m^{-2}) in proportion to the maximum plant density and (**A**) daily variations in rainfall (mm) for elevated zones, (**B**) daily variations in maximum air temperature (°C) in depressed zones, and (**C**) daily variation in soil conductivity (mS cm^{-1}) in depressed zones. Regression equations: (**A**) $y = 0.0031x - 0.0010$ ($R^2 = 0.084$, $p = 0.0058$, $n = 207$); (**B**) $y = -0.0372x + 0.0013$ ($R^2 = 0.104$, $p = 0.0005$, $n = 207$); (**C**) $y = -0.0037x - 0.0006$ ($R^2 = 0.066$, $p = 0.0218$, $n = 207$).

The density of the surviving plants at the end of the study increased together with maximum plant density in elevated and depressed zones (Figure 5A, Table 5), showing similar values in both (Tables 2 and 3). In relation to the soil-seed-bank density, the plant density at the end of the study represented 15 ± 12% for P1, 14 ± 4% for P2, 2 ± 1% for P3 and 4 ± 1% for P4, with no significant differences among populations (Kruskal–Wallis test: $H(3,12) = 5.36$, $p = 0.1473$). The proportion of surviving plants in relation to the maximum plant density varied between 11 ± 4% for P3 and 64 ± 10% for P2, being 58% greater in elevated zones than in depressed zones (Tables 2 and 3).

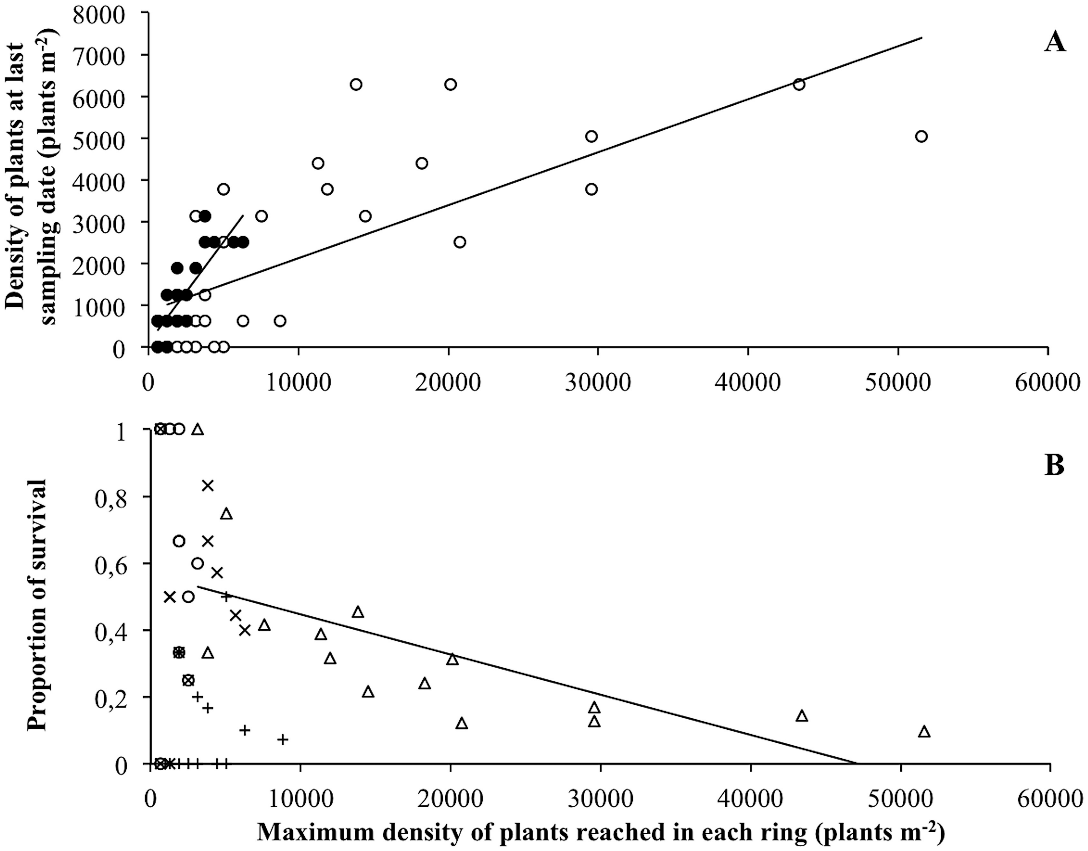

Figure 5. Relations between the maximum plant density reached in each sample ring and (**A**) plant density on the last sampling date in elevated zones (black circles) and depressed zones (White circles), and (**B**) the proportion of surviving plants in each sample ring in four *Salicornia ramosissima* populations (P1, cross; P2, circle; P3, plus; P4, triangle). Regression equations: (**A**) elevated zones, y = 0.482x + 108.790 (R^2 = 0.688, p < 0.0001, n = 23); depressed zones y = 0.127x + 861.010 (R^2 = 0.537, p < 0.0001, n = 30); (**B**) P4, y = -0.00001x + 0.567 (R^2 = 0.473, p < 0.0001, n = 15).

Table 5. Spearman correlation coefficent (ρ) and probability value (p) for correlations between the maximum plant density reached in each sampling ring and different population and plant characteristics for four populations of *Salicornia ramosissima*, and for populations in elevated and depressed zones. Significant values are marked in bold (p < 0.05)

Daily Variation in Plant Density in Proportion to Maximum Density vs.		1	2	3	4	Elevated Zones	Depressed Zones
Daily rainfall	r	**0.283**	0.083	**0.198**	0.020	**0.191**	0.098
	p	**0.0030**	0.4172	**0.0214**	0.8177	**0.0058**	0.109
Daily variation in maximum temperature	r	−0.020	−0.109	**−0.344**	−0.102	−0.061	**−0.210**
	p	0.8376	0.2851	**0.0000**	0.2399	0.3825	**0.0005**
Daily variation in minimum temperature	r	0.084	−0.012	**−0.259**	0.013	0.039	−0.118
	p	0.3876	0.9058	**0.0025**	0.8811	0.5761	0.0531
Daily variation in soil pH	r	−0.087	0.034	−0.037	**−0.223**	−0.017	−0.128
	p	0.4317	0.7692	0.7058	**0.0222**	0.8314	0.0636
Daily variation in soil conductivity	r	−0.070	0.060	**−0.230**	**−0.194**	−0.016	**−0.158**
	p	0.5274	0.6021	**0.0181**	**0.0471**	0.8411	**0.0218**

At the end of the study, the density of blooming and fruiting plants was similar in elevated and depressed zones. The proportion of blooming and fruiting plants in relation to the plant density at the end of the study was also similar between physiographic zones. In contrast, the proportion of blooming and fruiting plants in relation to the maximum plant density was 55% higher in elevated zones than in depressed zones (Table 2). Thus, the density of blooming and fruiting plants increased together with the maximum plant density in elevated zones and depressed zones (Table 5). Furthermore, the final proportions of surviving and blooming and fruiting plants decreased when the maximum plant density increased only for P4 (Figure 5B, Table 5). The plant height at the end of the study did not change between elevated and depressed zones (Tables 2 and 3).

5.5 Discussion

Our results show that the annual halophyte *S. ramosissima* presents contrasted population dynamics depending on the physiographic position in salt pans under a Mediterranean climate, in relation to differences in plant density, partially in agreement with our first hypothesis, and to the environmental matrix, refuting our second hypothesis.

In elevated areas with good drainage, *S. ramosissima* populations presented maximum plant densities of between 628 and 6288 plants m^{-2} that remained almost constant until fruiting. These maximum densities maintained a dynamic equilibrium due to new seedling establishments and plant survival rates of between 46–64%. This survivorship curve pattern, which could be assimilated to Type I of [37], reveals that the mortality of individual plants was concentrated at the end of their lifespan. In contrast, *S. ramosissima* populations colonizing frequently waterlogged depressed zones presented five-times greater soil-seedbank densities and maximum plant densities (between 1257 and 51,558 plants m^{-2}) than populations in elevated zones. These high seed-bank densities may be the result of higher in situ seed production [8] and the transport of seeds from elevated to depressed zones. Previous studies have reported a positive correlation between soil-seed-bank density and plant density for *S. ramosissima* [8] and *S. europaea* [19]. In our context, populations in depressed zones lost c. 60% of their maximum plant densities from the end of spring and throughout summer. This is a hot, dry period in the Mediterranean climate, when increasing air temperatures are related to high evapotranspiration rates that result in high sediment salinities [7,38] and high plant mortality in salt marshes [39]. Thus, we began to record plant mortality from when the maximum air temperature increased by 0.05 ºC or more daily, and from when the sediment EC started to increase. Rainfall and sediment moisture, and salinity, have been reported as the major environmental factors controlling establishment and death, respectively, in different *Salicornia* species [20,21,29,32,40]. The lowest EC recorded in our study was c. 10–20 mS cm^{-1} in winter and early spring, which correspond to salinities c. 200 mM NaCl that are close to the optimum growth range recorded for *S. europaea* (between 200–400 mM NaCl) [41]. The highest EC was c. 60–80 mS cm^{-1} in late spring and summer, corresponding to c. 600–800 mM NaCl, which are values that have been reported as growth-limiting for *S. europaea* (>400 mM NaCl) [41]. The survivorship curve pattern for *S. ramosissima* in the depressed zones could be assimilated to Types II or III of [34] and describes a situation in which individuals are affected by high mortality rates from the beginning of their adult stage. Similar curves were observed by [25] for populations of *S. ramosissima* with densities of 3000–9000 plants m^{-2} in salt pans in the Odiel Marshes. Moreover, [19] observed Type II surviving curves for *S. europaea* on the coast of Norfolk (England), stating that the proportion of plants that died before flowering depended on environmental conditions rather than on seedling density per se. Similarly, [33] indicated that abiotic stress was the primary cause of mortality in *S. europaea*, since its survival was not related to peaks in plant density as high as 65,000 plants m^{-2}. In whatever way the environmental conditions seemed to control the population dynamic of *S. ramosissima* in depressed zones, the effects of a stressful environment would interact with plant densities since sediment EC and air temperatures were similar between physiographic locations, but plant densities were higher in depressed zones than in elevated zones. Along these lines, [42] registered a Type I survivorship curve for a density of 261 plants m^{-2} and a Type II survivorship curve for denser populations of *S. ramosissima* in the Aveiro Lagoon (Northwest Iberian Peninsula), proposing that the combined effect of sediment salinity and competition could cause the abrupt decrease in plant density observed in dense populations of *S. ramosissima* (c. 1700 plants m^{-2}). Besides the contrasted population dynamics recorded in our study and that previous works have reported local adaptation to salinity for different *Salicornia* populations [43], in our case, it did not seem that there was local adaptation since the populations of elevated and depressed areas were very close to each other, so there would be genetic exchange between them, both through pollen and seeds.

Contrary to our first hypothesis, we found density-dependent mortality for the densest population (P4; max. 51,558 plants m^{-2}). This result contradicts the density-independent seedling mortality recorded for *S. europaea* in Northern European marshes where the environmental matrix is more benign [19, 22–24]. In this regard, [44] designed a field study on the North Atlantic Coast of North America comparing *S. europaea* responses at different individual densities, from 100 to more than 10,000 plants m^{-2}, and concluded that plant density affected plant biomass and morphology, but not mortality. Thus, the general principle for halophyte survival states that mortality is mostly influenced by abiotic stresses rather than by plant densities [45]. However, some exceptions have already been established, such as *Spergularia marina* (L.) Griseb. [46].

Besides these contrasted responses in population dynamics that depend on physiographic position in the salt pans, all *S. ramosissima* populations concentrated the establishment of new seedlings during winter under a Mediterranean climate, when sediment salinity reached the lowest values. In this sense, *S. ramosissima* presents its maximum germination rates in fresh water after exposure to high salinities (0.6–0.9 M) [18]. Taking advantage of germination windows is especially important for halophytes colonizing highly stressful habitats such as salt pans [47–49]. In addition, air temperature plays an important role in the germination of *S. ramosissima* given that its seedling establishment occurred mostly from March to May in the Northwest Iberian Peninsula [42], where air temperatures are lower than in the Odiel Marshes. Low temperatures inhibit the germination of different *Salicornia* species [29,50]. On the other hand, maximum plant densities represented between 14–31% of the seed-bank density at both physiographic positions. [51] reported maximum plant densities representing between 10–59% of the seed-bank density for *S. procumbens* and c. 41% for *S. europaea* in The Netherlands. Our results show that *S. ramosissima* retained viable non-germinated seeds after the maximum peak of seedling establishment. In fact, we recorded increases in plant density during spring and summer after maximum plant densities were reached. This temporal germination strategy at the population level was probably based on a staggered breaking of the physiological dormancy induced by high salinities [17, 18]. Therefore, this temporal pattern of germination may allow some *S. ramosissima* plants to avoid catastrophic events, such as herbivory or violent river floods, that could impact its populations throughout the growing season. Even so, the *S. ramosissima* seed bank was drastically reduced during the first year after seed dispersal [8], as occurred in *S. europaea* [19]. Additionally, all study populations presented similar plant height that was unrelated to plant density, so it seems to be linked to particular habitat characteristics or/and genetic differentiation, as demonstrated in *S. europaea* using transplant experiments [23,27,32].

In view of our results, climate change, which causes sea level rise [52] and reduces rainfall in the Mediterranean Basin [53], would increase sediment salinity in the salt pans thereby reducing the germination and establishment of *S. ramosissima*. This reduction in seedling establishment may reduce the final densities of fruiting plants in elevated zones and, at the same time, may lessen the density-dependent dynamic in depressed zones. In this scenario of climate change, halophytes offer an exceptional opportunity for saline agriculture [5]. Thus, our results are useful for planning the sustainable harvesting of natural populations of *S. ramosissima*. For example, plants could be harvested at the end of winter or the beginning of spring in depressed areas where plant densities would be greater than c. 15,000 plants m^{-2}, which would help to avoid density-dependent mortality. Concrete harvest strategies should be designed for each population of *Salicornia* since they inhabit different environmental conditions and show high genetic and morphological variability [54]. In this context, our results are useful for the protection, restoration and sustainable exploitation not only of coastal salt marshes, but also of inland salt marsh habitats colonized by *Salicornia* species [55].

5.6 Material and methods

5.6.1 Study area

The present study was carried out in tidal salt marshes of the Odiel Natural Park (37º 12´ 32.3´´ N, 6º 58´ 01.5´´ W) located in the Gulf of Cádiz (Southwest Iberian Peninsula). The Odiel Marshes are one of the largest areas of salt marshes in the Iberian Peninsula and are protected as a Natural Reserve of the Biosphere by UNESCO. The study area has a semi-diurnal mesotidal regime, with a tidal range (equinoctial mean) of 2.97 m [56]. The Odiel Marshes are subjected to a Mediterranean climate with Atlantic influence [57]. Annual mean air temperature is 18 ºC and mean monthly temperatures range between +11 ºC in January and + 25 ºC in August. Annual average precipitation is 523 mm, with a 4–5 month dry period from approximately June to September (data series 1984–2010 from the meteorological station at Francisco Montenegro Avenue, in the city of Huelva, located close to the marshes under study (37º 14´ 29´´ N, 6º 56´ 55´´ W).We sampled four populations of *S. ramosissima* in two elevations in two different salt pans: two populations (P1 and P2) located in elevated areas that were rarely inundated, and another two populations (P3 and P4) colonizing depressed areas that were usually waterlogged. The elevation difference between these two physiographic positions was c. 20 cm. Previously, we characterized these two physiographic positions in another study on the *S. ramosissima* seed bank [8]. P1 and P3 were located in an abandoned saltwork evaporation pond (37º 15´ 41.6´´ N, 6º 58´ 35.54´´ W). P2 and P4 colonized salt pans (P2: 37º 13´ 39.63´´ N, 6º 57´ 46.52´´ W; P4: 37º 13´ 34.74´´ N, 6º 57´ 50.54´´ W).

5.6.2 Meteorological data

Daily mean, maximum and minimum air temperatures and precipitation during the study period were obtained from the meteorological station at Francisco Montenegro Avenue. We calculated daily variations in air temperature for a certain sampling period as the temperature difference between two consecutive sampling dates divided by the number of days of that period. Daily rainfall for a sampling period was calculated by dividing the total rainfall accumulated since the previous sampling date by the number of days of the period.

5.6.3 Sedimentary environment

We randomly choose three zones in each study population of *S. ramosissima*. In each zone, we took three sediment samples on 12 December 2019, 1 January, 13 February, 11 May, 10 June, 24 June, 10 July, 4 August and 11 September 2020, resulting in a total of 324 samples. Sediment samples were randomly collected using stainless-steel cores of 50 mm diameter and 50 mm height. Samples were placed in hermetically sealed polyethylene bags and stored at -20 ºC until laboratory analysis. Sediment electrical conductivity (EC) and pH were measured in the unfiltered supernatant of a homogenized mix of 5 ml of wet sediments and the same volume of distilled water (1:1, v:v) using a conductivity meter, Crison Instruments 5064 (Hach Lange Spain, S.L.U., Barcelona, Spain), and a Crison pH meter 25 (Hach Lange Spain, S.L.U., Barcelona, Spain) [58]. We calculated daily variations in EC and pH for a sampling date by subtracting the value obtained in the previous sampling from the value obtained on the current date, divided by the number of days between these sampling dates. Field sediment salinity was calculated from electrical conductivity following this equation: Salinity (ppm NaCl) = EC (µS cm^{-1})x0.46 [59].

5.6.4 Soil seed bank of *Salicornia ramosissima*

The soil seed banks were studied at the beginning of the study, on 12 December 2019. We took 15 soil samples per population, 5 at each of three randomly selected zones, using stainless-steel cores of 50 mm diameter and 50 mm height. Samples were placed in hermetically sealed polyethylene bags and transported to the laboratory for analysis. Sediment samples were sieved through a 0.4 mm light sieve to eliminate most of the clay matrix, and the material that remained in the sieve was examined under a magnifying glass to look for seeds [60]. We calculated the density of seeds (seeds m^{-2}) for each population.

5.6.5 Population dynamic of *Salicornia ramosissima*

On 12 December 2019, we placed 15 plastic rings of 4.5 cm diameter around *each S.ramosissima* population, 5 at each of three randomly selected zones. These rings were labeled and anchored to the ground using stakes. Each *S. ramosissima* plant growing within each ring was counted on 12 December 2019, 1 January, 13 February, 11 May, 20 May, 10 June, 24 June, 10 July, 4 August and 11 September 2020. On 11 September 2020, we counted those plants that were blooming or presented ripening fruits and recorded the height of every *S. ramosissima* plant inside all the sampling rings, measuring from the sediment surface to the highest plant tip. Seven sampling rings in which no plant was observed during the study were discarded from analyses. Signs of predation on some parts of flowering or fruiting plants of *S. ramosissima* were only sporadically observed after the last sampling date.

We calculated the plant density per surface unit for each sampling ring at each sampling date. Then, we established the sampling date in which the highest density value was reached and the value of this maximum plant density. With these data for each sampling ring, we calculated the final proportion of surviving plants as plant density on the last sampling date (11 September 2020) in relation to the maximum plant density reached for each ring. We also calculated the proportion of blooming plants in relation to the total plant density on the last sampling date, and to the maximum plant density reached. In addition, we calculated the daily variation in plant density relative to the maximum density for each sampling ring on each sampling date, as the difference between the numbers of plants on two consecutive sampling dates divided by the maximum density reached and by the number of days between those sampling dates.

5.6.6 Data analyses

Analyses were carried out using Statistica 8.0 (StatSoft INC., USA). Deviations from the mean were calculated as standard error (SE). Significant differences were considered when $p < 0.05$. Data or their transformations ($\log(x + 1)$, $1/(x + 1)$ and $\sqrt{x}$) were tested for homogeneity of variance and normality with the Levene test and the Kolmogorov–Smirnov test, respectively. None of the tested data series followed normal distribution; therefore, the Kruskal–Wallis test was used to compare significant differences between the means of the four study populations; the Mann–Whitney U test was applied to detect significant differences between the means in elevated and depressed areas. The non-parametric Spearman's correlation coefficient (ρ) was used to analyze the relationships between sedimentary variables and the recorded variables for *S. ramosissima* plants.

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