Salinity as the major factor affecting *Scirpus maritimus* annual dynamics
Evidence from field data and greenhouse experiment

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Abstract

During the life cycle of *Scirpus maritimus*, ramets produce photosynthetic shoots that are active for a single growing season and only the belowground parts persist into the next year. Several authors have described that the growing season of *S. maritimus* in Europe and North America occurs between April and September of each year. In the Mondego estuary (western coast of Portugal) the life cycle of *S. maritimus* showed an opposite seasonal pattern, with a growing season from January to April/May.

It was hypothesised that seasonal fluctuations in salinity, connected to salinity tolerance, could explain this contrasting annual cycle. This hypothesis was tested using a greenhouse experiment.

Exposure to different salinities significantly affected plant survival. During the first 10 days, the survival curves were very similar, which suggested that the plants were able to tolerate high salinity for short periods. After 2 weeks of exposure, the plants started to show differences, with increased mortality following the increase in salinity.

Results show that a peculiar seasonal variation of salinity in the Mondego estuary affected the *S. maritimus* life cycle in this ecosystem and may explain the differences between these western Portuguese populations and other European and North American populations.

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1. Introduction

In the Mondego River estuary (Fig. 1) on the western coast of Portugal, *Scirpus maritimus* salt marshes occupy the intertidal mud flats in the upstream areas of the southern
arm (Lillebø et al., 2002; see also Flindt et al., 1997; Pardal et al., 2000; Martins et al., 2001). This species is widely distributed in Europe (Peláez et al., 1998) and North America (Karagatzides and Hutchinson, 1991; Kantrud, 1996) and it usually forms dense monospecific stands in shallow brackish marshes (Charpentier and Stuefer, 1999). The growth of *S. maritimus* stands can be divided into three phases: (i) a juvenile phase; (ii) a mature phase, with higher shoot density and height and (iii) a senescent phase, with a dominance of belowground plant parts (Clevering and Van der Putten, 1995). In this species, photosynthetic shoots are active for a single growing season and only belowground parts persist into the next year (Charpentier and Stuefer, 1999). In most of the fresh water and brackish water systems studied by other authors, the growing season of *S. maritimus* occurs between April and September (e.g. Karagatzides and Hutchinson, 1991; Clevering, 1995; Kantrud, 1996; Clevering and Hundscheid, 1998; Charpentier and Stuefer, 1999). In the Mondego estuary however, *S. maritimus* start the juvenile phase in late December/January and the mature phase in March/April/May, while the standing dead biomass follows in the summer and fall periods.
Based on data from the Mondego monitoring program (1996–2000, e.g. Martins et al., 2001; Lillebo et al., 2002), from the Scirpus field survey (1997–1998) (Lillebo et al., 2002) and data from other systems, we hypothesised that salinity could play a major role in the annual dynamics of this species. This hypothesis was tested by performing a greenhouse experiment of 6 weeks where plants were exposed to different concentrations of salinity at a constant temperature, both representative of field values.

2. Materials and methods

2.1. Mondego estuary monitoring program

The Mondego estuary has been monitored since 1993. Data on water temperature, precipitation and salinity were collected by several authors in one common area in the south arm of the Mondego estuary (Fig. 1) (e.g. Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2002; Tillebo et al., 2002) and are a part of the Institute of Marine Research (IMAR) data base.

2.2. Scirpus field survey

At three sites (Fig. 1) located in the upstream areas of the southern arm of the estuary, plants were collected at monthly intervals (March 1997 to June 1998), from stands that looked uniform in size and density of stems. At each study site, five random cores were taken (141 cm² in area) and all the aboveground material was clipped to ground level. The dry mass of live and dead shoots was quantified separately. At each station, salinity, temperature and pH were measured in situ in low water pools. Sediment sub-samples were taken in triplicate and analysed for dry weight and loss of weight after ignition (8 h, 450 °C). The granulometry was analysed seasonally and classified according to the nomenclature proposed by Brown and McLachlam (1990).

2.3. Greenhouse experiment

Adult plants of *S. maritimus* corresponding to the mature phase were collected in the Mondego estuary salt marsh (study site 1) on 11 April 2000 (Fig. 1). Whole green plants were gently washed with estuarine water to clean the sediment from the rhizome and roots and were brought to the laboratory. All plants (one shoot plus rhizome) were placed under similar conditions: in pots containing sand (particle size > 1 mm) and water with the same nutrient concentration and salinity. The sand was previously washed and sterilised at 500 °C for 3 h to avoid contamination by salt and organic matter. Artificial pond water (Canhoto and Graça, 1999) was prepared in the laboratory and the salinity was adjusted to 6‰ S with marine salt, which corresponded to the mean salinity value in the sediment when the plants were collected. The water level was maintained at 1 cm above the substratum.

The experimental set-up included 24 pots (Ø 16 cm and a volume of 3.2 l) with a set of five plants in each. All plants were placed in a greenhouse with natural sunlight and constant temperature (25 °C). All pots contained the same nutrient concentration (620 mg
N/l and 94 mg P/l) and salinity was kept constant by watering the pots with distilled water to compensate for evaporation. After the first 2 weeks, all pots had at least one or two plants with clear signs of both senescence and emergent new shoots. The pots were maintained under the initial conditions and the experiment began after the development of the new shoots. Each pot had at least four new shoots and after 4 weeks since the emergence of new shoots, the new stems were about 10 cm height.

On 19 May, the salinity was adjusted to six different concentrations of 0, 10, 15, 20, 25 and 30% S. Four pots were randomly allocated to each treatment and four plants, corresponding to the new emergent shoots in each pot, were monitored. To account for pseudoreplication (Hurlbert, 1984), the experimental unit was the pot and not each of the four plants (actually shoots measured) per pot. Salinity was measured every 3 days and when necessary, distilled water was added to compensate for evaporation. Due to this procedure, pH was also measured after each watering. Stem length was measured every 3 days and growth rates calculated as differences in plant size over time. Simultaneously, plants were inspected regularly for any signs of senescence that was recorded as the percentage of necrotic tissue in aboveground material. Plants were considered as dead standing biomass when the aboveground part had 100% of tissue necrosis. Survivorship curves were compared using the nonparametric Gehan-Wilcoxon test for censored data (Pyke and Thompson, 1986).

3. Results

3.1. Mondego estuary monitoring program

The Mondego estuary showed a quite constant inter-annual pattern of water temperature between October 1996 and September 2000 (Fig. 2). Precipitation rates were quite variable, which had a strong influence on the salinity (Fig. 2). During a rainy winter, salinity values were around 10% S (1997 and 1998), but these increased to 25% S during a dry winter period (1999 and 2000). Between October 1998 and September 1999, because precipitation was low, the salinity values were comparatively high, although water temperature showed the normal pattern. During this one season period, S. maritimus populations presented two generations. New shoots emerged in December (light grey bar), but in March these had withered into dead standing biomass (black bar) and new shoots started to emerge again (light grey bar, Fig. 2).

3.2. Scirpus field survey

At S. maritimus salt marshes, the sediment mainly consisted of fine particles (63–125 μm), corresponding to salt and clay.

Salinity and temperature measured in the salt marsh low tide pools (Fig. 3) followed the annual pattern already described for the estuary (Fig. 2). In the Mondego estuary (Fig. 3), S. maritimus new shoots emerge in late December/January. The plant mature phase occurs in March/April/May (living shoots) and the senescent phase (dead standing biomass not plotted) is in the summer period. Spatial differences were observed in salinity and aboveground
Fig. 2. (a) Inter-annual variation of water temperature; (b) precipitation; (c) salinity in the Mondego estuary (n = 4, mean monthly data ± S.E.), from October 1996 until September 2000 and *S. maritimus* life cycle (authors observation), from January 1997 until September 2000—salinity in white bars; emergence of *S. maritimus* new shoot in light grey bars; standing dead biomass in black bars; (*) : no data available.
plant biomasses. Greater plant biomass was achieved in the most inner areas of the estuary (study site 3, Fig. 3c), where the lowest salinities were recorded (Fig. 3a) and the lowest biomass in the more downstream areas of distribution (study site 1, Fig. 3c), where the highest salinities were recorded (Fig. 3a). Tidal pools pH in the water pools and organic matter content of the sediment showed little temporal variation at all three study sites. From study site 1 to the inner areas, the water pH (mean ± S.D.) was 8.03 ± 0.34, 7.90 ± 0.63
and 8.05 ± 0.56, respectively and the percentages of organic matter in the sediment were 9.19 ± 1.4, 7.99 ± 0.98 and 10.20 ± 1.96, respectively.

3.3. Greenhouse experiment

The exposure of *S. maritimus* to different salinities for 1.5 months, significantly affected the mortality of the plants (Fig. 4a). During the first 10 days, the survival curves were very similar among treatments, which suggests that the plants were able to tolerate high salinity environments for short periods. After 2 weeks of exposure, the plants started to show differences, with increased mortality following the increased exposure to higher salinities. Considering the survival curves among treatments (Fig. 4a), the Gehan–Wilcoxon test showed that there are three statistically different groups (*P* < 0.001; labelled with different letters). The first group includes salinity values of 0, 10 and 15% S (no significant differences among them—c curve), the second group includes 20 and 25% S (no significant differences among them—b curve) and the third group corresponds to a salinity of 30% S (a curve).

![Fig. 4](image-url)
During the experiment _S. maritimus_ also showed differences in stem elongation when exposed to different salinities (Fig. 4b). Throughout the period of exposure to different salinities, plants stem elongation decreased with increasing salinity. Although there were no significant differences among the survival curves of the plants exposed to salinities of 0, 10 and 15% S, stem growth rates were significantly different (Tukey test, \( P < 0.001 \)). Additionally, new shoots emerged during the entire experimental period (not counted or measured), but only in the pots with freshwater, suggesting that low salinity values (<10% S) were needed for the emergence of new shoots. The pH values in the pots ranged between 7.9 and 8.2, which falls within the range of pH field data.

4. Discussion

We observed a substantially lower biomass at the station experiencing the highest salinity in February/March 1998 (site 1), which is in agreement with our hypothesis. Furthermore, our experiment showed that survival was severely affected by salinities higher than 15% S and that salinity strongly restricted plant growth, equally supporting our hypothesis. Hence, seasonality in salinity strongly conditioned the plant growth cycle. These results are in agreement with Hootsmans and Wiegman (1998) and suggest that _S. maritimus_ is able to tolerate high salinity environments but only for short periods and that plant senescence will be induced after a longer exposure to salinity values higher than 15% S. This species has been classified as a facultative halophyte of the accumulating type (Kantrud, 1996). In this species, leaves present some adaptations to survive under saline conditions (Mateu, 1991), but high salinity reduces the overall plant growth rate (Hootsmans and Wiegman, 1998), conditions the emergence of new shoots (Kantrud, 1996) and may also affect seed germination and seedling survival, as well as the production of established populations (Lieffers and Shay, 1982a,b). Moreover, in estuaries stands of _S. maritimus_ usually develop best in the mesohaline portions where fresh water flows periodically (Kantrud, 1996).

In our results, the mean aboveground biomass of _S. maritimus_ in study site 1 falls within the range of the values referred by Karagatzides and Hutchinson (1991) for temperate coastal marshes in North America and by Lieffers and Shay (1983) for two ephemeral saline lakes on the Canadian prairies. Our findings in study sites 2 and 3 (the inner stations, experiencing lower salinity) correspond to the range of values referred by Dykyjova (1971) for natural stands of emergent macrophytes in southern Bohemia, Czech Republic.

Salinity in salt marsh sediments is conditioned by evaporation, tidal flooding, precipitation rates and salinity of tidal waters (Adams and Bate, 1995). In the Mondego estuary southern arm, the inflow and outflow regime depend essentially on tides and a small tributary, the Pranto River, which is controlled by a sluice according to the water needs for rice fields at the Pranto Valley (Flindt et al., 1997; Marques et al., 1997; Lillebø et al., 1999; Pardal et al., 2000; Martins et al., 1999, 2001). During the winter period, the freshwater runoff from the Pranto River will increase and consequently salinity values are lower. But, although there might be high precipitation rates during spring, salinity usually increases, because water is kept behind the sluice, due to the need of water for the rice fields (Martins et al., 2001). As a result of this peculiar seasonal variation, winter seems to be the only time of the year when salinity values are usually low. Moreover, results also suggest that salinity values
below 10% S are needed for the emergence of new shoots. On the other hand, due to its
geographical location, the Mondego estuary is characterised by a warm temperate climate,
which may explain why water temperature during winter does not condition the emergence
of new shoots (see field monitoring data). Finally, apparent day length and light availability
in winter do not affect growth negatively.

Our results show that the seasonal salinity variation in the Mondego estuary may condition
two main periods of the *S. maritimus* life cycle, which explains the differences between this
system and others across Europe and North America. Firstly, the winter period is the only
time of the year when salinity values are below 10% S, which seems to be needed during the
emergence of new shoots. Secondly, the continuous exposure to increasing spring salinities
(above 15% S) induces the senescence of plants.

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**References**


