Ecophysiology of tidal and non-tidal populations of the invading cordgrass *Spartina densiflora*: seasonal and diurnal patterns in a Mediterranean climate

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Abstract

The invasion of salt marshes by alien species poses interesting questions concerning the mechanisms that determine their distribution. *Spartina densiflora* is one of the species that is invading the Atlantic marshes of SW Iberian Peninsula, where it tolerates a broad range of environmental conditions and it competes with the indigenous vegetation. The high ecological versatility of *S. densiflora* provides a good tool to study the ecophysiological responses of marsh plants. This article aims to compare the ecophysiological responses of *S. densiflora* in populations from two contrasting habitats: tidal (middle marsh) and non-tidal (drainage intercepted marsh). The higher values in \(A\) (net photosynthesis rate), \(A/C_i\) (apparent carboxylation efficiency), water use efficiency (intrinsic WUE) and \(F_v/F_p\) (potential photochemical efficiency) recorded at the non-tidal population, except in summertime, agree with previous studies that found better physiological state at lower salinity and higher redox potential. A clear reduction of \(A\) at higher irradiance was recorded in spring and summer in the tidal population, coinciding with the lowest \(w\) (leaf water potential) values. These results help to explain the high primary productivity recorded in *S. densiflora* populations at brackish marshes in Mediterranean areas. Different responses in gas exchange and chlorophyll fluorescence during summer were recorded for each population. The tidal population showed the maximum values of \(A\) in summer. In contrast, the non-tidal population suffered reductions in \(A\), \(A/C_i\) and \(F_v/F_p\) during summer, when salinity was higher. Thus, salinity limits carbon fixation in *S. densiflora* non-tidal populations during Mediterranean summer drought. In tidal populations, photosynthesis seems to be more influenced by anoxic conditions. High levels of photoinhibition and low \(A\) were recorded on the coldest and less cloudy day, which provoked permanent damages to the photosynthetic apparatus of *S. densiflora*. This may limit its winter production, as well as its invasion of marshes at higher latitudes in Europe. Finally, discussion about the possible effects of Global Climatic Change on *S. densiflora* invasion is undertaken.

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**Keywords:** invasive *Spartina*; marshes; global Climatic Change; gas exchange; chlorophyll fluorescence; leaf water potential; SW Spain

1. Introduction

The invasion of salt marshes by alien species of high competitive potential poses interesting questions concerning the mechanisms that determine their eventual distribution and their effects on vegetation zonation (Castillo et al., 2000). *Spartina densiflora* Brong. is one of these species (Kittelson & Boyd, 1997; Nieva, Díaz-Espejo, Castellanos, & Figueroa, 2001). South American in origin (Mobberley, 1956) it is invading the Atlantic marshes of the Gulf of Cádiz (SW Iberian Peninsula), from San Vicente Cape (Portugal) to Gibraltar (Nieva, Castellanos, & Figueroa, 2002).

Different species of the genus *Spartina* have specialised in tolerating certain levels of tidal submergence; however, *S. densiflora* is able to encompass a broad range of habitats between the lowest and highest levels of salt marshes. In some salt marshes in the Gulf of Cádiz,
**2. Materials and methods**

### 2.1. Study area

The study was conducted on the Odiel marshes, in the joint estuary of the Odiel and Tinto rivers, SW Spain (37°08’–37°20’N, 6°45’–7°02’W). These are among the most extensive and diverse salt marshes of the Iberian Peninsula. The tide is semi-diurnal and the tidal range (mean spring) is 2.97 m (0.4–3.37 m above hydrographic zero). The climate is Mediterranean with Atlantic influence (Marine Mediterranean Climate). More detailed descriptions of these marshes are given by Castellanos, Figueroa, and Davy (1994) and Nieva and Luque (1996). Nomenclature was according to Tutin et al. (1968–1993).

Meteorological data during the study were supplied by the ‘Estación Meteorológica de Huelva’, the nearest meteorological station to the Odiel marshes (Fig. 1).

Work was carried out in two different populations. One located on a middle marsh near to a tidal channel (MM) and the other one on a high marsh not influenced by the tide (DIM) because its drainage has been intercepted after the construction of the Juan Carlos I Dike in 1977. Abiotic characteristics of the sediment, distribution of above and below-ground vegetative structures and rate of flowering in both populations have been previously described by Nieva et al. (2001). Table 1 summarises some of these characteristics registered at the beginning of autumn.

### 2.2. Leaf gas exchange

The field work was done during 7 days (never on neap tides) (at middle marsh, MM), and 8 days (at high marsh, DIM), between July 1993 and June 1994. Diurnal curves were run on each sampling day, with measurements made at 2 h intervals from early morning to late afternoon. Records were made on intact, young, fully expanded, leaves \((n = 8–10)\) from the same number of randomly selected tillers of four to five different

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**Fig. 1.** Monthly mean maximum (filled circles) and minimum (open circles) temperatures, and monthly precipitation (bars) during the study at Odiel marshes.
tussocks of *S. densiflora*. When the relative humidity in the early morning was 80% or higher, measurements were postponed in order to avoid anomalous functioning of the infrared CO₂ analyser.

Measurements of net photosynthesis rate \( \left( A \right) \) and stomatal conductance rate \( \left( gs \right) \) were made using a portable open system with infrared CO₂ analyser (model LCA-3, Analytical Development Co.) in differential mode, equipped with an ADC Parkinson leaf chamber (model PLC-3N); relative humidity and temperature in the leaf chamber were maintained similar to that prevailing outside. Dates and environmental conditions during the sampled days are shown in Table 2.

Sub-stomatal (intercellular) CO₂ concentration \( \left( C_i \right) \) was calculated using conventional formulae (Pearcy, Ehleringer, Mooney, & Rundel, 1989). Intrinsic water use efficiency (WUE) was calculated as \( \frac{A}{gs} \) and apparent carboxylation efficiency as \( \frac{A}{C_i} \) (Flexas, GulliÁE, Jonasson, Medrano, & Mus, 2001).

### 2.3. Chlorophyll fluorescence

The chlorophyll fluorescence fast kinetics of intact young leaves of both populations were recorded at the same time as gas exchange measurements, using a portable non-modulated fluorimeter (Plant Stress Meter, PSM Mark II, Biomonitor S.C.I.). Details of the instrument are provided by Öquist and Wass (1988). Fluorescence determinations \( \left( n = 8–10 \right) \) were made at the prevailing air temperature. Leaves were adapted to darkness using white leaf-clips (Biomonitor 1020) for 30 min before measurement of the fluorescence transient over 2 s.
Initial fluorescence ($F_0$) was determined by the shutter aperture, which proved fast enough to give satisfactory resolution. At this moment, the state of opening of the photosystem II (PSII) reaction centres was maximal and therefore the rate of photochemical processes was not limited. $F_0$ depends on the size of the PSII chlorophyll antenna and on the functional integrity of PSII reaction centres (Krause & Weis, 1991). A flash of actinic light yielded a peak of fluorescence ($F_p$). The half-time for transition from $F_0$ to $F_p$ ($T_{1/2}$) was determined and it is related with the reduction rate of the PSII electron acceptors quinone A (Q$_{A}$), quinone B (Q$_{B}$) and plastoquinone (PQ). It has been used to determine the capacity of functional PSII centres and the size of the PQ pool (Bolhär-Nordenkampf, Hofer, & Lechner, 1991; Bolhär-Nordenkampf & Öquist, 1993). The ratio of variable fluorescence ($F_v = F_p - F_0$) to $F_p (F_v/F_p)$ was used as a measure of maximum photochemical yield of PSII; this ratio correlates with the number of functional PSII reaction centres (Öquist, Chow, & Andersson, 1992) and was used to quantify photoinhibition (Osmond, 1994) and stress (Lichtenhaler, 1988).

### 2.4. Leaf water potential

Leaf water potential ($\psi$) was measured simultaneously with gas exchange and chlorophyll fluorescence in five young leaves of S. densiflora. The value of $\psi$ was obtained according to Schölander, Hammel, Bradstreet, and Hemmingsen (1965) using a pressure bomb (Manofrígido, Lisbon).

### 2.5. Statistical analysis

Analyses of variance (ANOVA) were carried out to reveal differences in physiological responses between the two populations and seasonal differences within populations. Tukey’s HSD test ($p < 0.05$) was calculated only when $F$-test was significant at the 0.05 level of probability. Before the analyses, data were tested for homogeneity of variance with the Levene test, and in cases of heterogeneous variances suitable data transformations were applied. All analyses were performed using Statistica release 5.1 (Statsoft Inc.).

### Table 3

<table>
<thead>
<tr>
<th>A</th>
<th>WUE</th>
<th>A/C$_i$</th>
<th>$\psi$</th>
<th>$F_v/F_p$</th>
<th>$F_0$</th>
<th>$T_{1/2}$</th>
</tr>
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<tbody>
<tr>
<td><strong>F</strong></td>
<td><strong>p</strong></td>
<td><strong>F</strong></td>
<td><strong>p</strong></td>
<td><strong>F</strong></td>
<td><strong>p</strong></td>
<td><strong>F</strong></td>
</tr>
<tr>
<td>MM</td>
<td>12.71</td>
<td>&lt;0.001</td>
<td>2.51</td>
<td>&lt;0.05</td>
<td>7.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DIM</td>
<td>26.32</td>
<td>&lt;0.001</td>
<td>1.9</td>
<td>n.s.</td>
<td>8.16</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3. **Results**

3.1. **Seasonal patterns (mid-day measurements)**

3.1.1. **Gas exchange**

Net photosynthesis ($A$) at mid-day was higher in DIM than in MM throughout the year, except during the summer drought (Tukey’s HSD Test, $p < 0.05$). The two S. densiflora populations showed opposite seasonal patterns of $A$. In MM, $A$ decreased progressively throughout the autumn, winter and early spring from a significant peak in August (12.35 μmol CO$_2$ m$^{-2}$ s$^{-1}$), increasing in late spring. In DIM, minimum values of $A$ were recorded during summer (4.27 μmol CO$_2$ m$^{-2}$ s$^{-1}$) and it tended to increase throughout fall and winter, showing its maximum during spring (15.37 μmol CO$_2$ m$^{-2}$ s$^{-1}$) (Fig. 2a).

Intrinsic WUE was higher in DIM ($p < 0.05$), except in August and January when it reached similar values in both populations (Fig. 2b). WUE, like gs, did not show a clear seasonal pattern. Annual variations in WUE were significantly different throughout the year only in MM (Table 3). The apparent carboxylation efficiency ($A/C_r$) followed closely the seasonal variation in $A$, reflecting few variations in $C_i$ (Fig. 2c).

3.1.2. **Chlorophyll fluorescence**

The mid-day $F_v/F_p$ in DIM showed a clear seasonal pattern, with maximum values during winter (0.714) and minimum in summer (0.555); this pattern was not detected in MM, which showed significant seasonal differences (Table 3), with maximum annual values in December (0.66) and the minimum (0.47) in the coldest sampled day (January) (Fig. 3a). Minimum $F_0$ values were registered in early winter in both populations, but a clear pattern was not observed (Fig. 3b). $T_{1/2}$ was maximum in early winter and summer in MM, showing its minimum in a foggy sampled day in August; in DIM minimum values of $T_{1/2}$ were recorded during summer (Fig. 3c). Differences between populations were significant only in November and June ($p < 0.05$), when MM presented higher values.

3.1.3. **Leaf water potential**

Mid-day leaf water potential ($\psi$) was lower at MM (between −5.1 and −8.1 MPa) than at DIM (between −3.7 and −7.1 MPa) ($p < 0.01$) (Fig. 4). Seasonal pattern
3.2. Diurnal patterns

3.2.1. Gas exchange

Usually, A increased in the morning, reached its maximum near noon, to decline thereafter in both sites; this pattern followed the diurnal course of the incident PAR. However, during summer and warmer days of the spring, A did not increase or even declined slowly during the central hours of the day (Fig. 5). This pattern was not seen in MM during August in a foggy day, which could be associated with the high relative humidity. Diurnal courses of WUE and A/C\textsubscript{i} were similar to those for A (data not shown).

was similar in both populations: the minimum was recorded during summer and the maximum in winter.

Fig. 2. Annual variations of mid-day net CO\textsubscript{2} assimilation (A) (a), intrinsic WUE (b) and apparent carboxylation efficiency (A/C\textsubscript{i}) (c) in tidal (○) and non-tidal (○) populations of S. densiflora. Data are means of 8–10 replicates ± SE. Different letters show significantly different levels within each population, with capital letters used for tidal population and lower case letters for the non-tidal population.

Fig. 3. Annual variations of mid-day F\textsubscript{v}/F\textsubscript{p} (potential photochemical efficiency) (a), F\textsubscript{0} (basal fluorescence) (b) and T\textsubscript{1/2} (half-time of transition from F\textsubscript{0} to F\textsubscript{p}) (c) in tidal (○) and non-tidal (○) populations of S. ensiflora. Data are means of 8–10 replicates ± SE. Different letters show significantly different levels within each population, with capital letters used for tidal population and lower case letters for the non-tidal population.
3.2.2. Chlorophyll fluorescence

Diurnal patterns of $F_{V}/F_{P}$ were similar in both sites, with maximum values recorded in early morning that decreased during the day, showing a partial recovery in the evening (Fig. 7). This general trend was registered every sampling day, except during January in MM, when the minimum values for this study were recorded (between 0.45 and 0.55) together with the absolute minimum temperature (Table 2).

3.2.3. Leaf water potential

Diurnal trends in leaf water potential were similar to those registered in $F_{V}/F_{P}$ in both populations. The maximum rates were recorded at the beginning of the day, decreasing to the minimum at mid-day and afternoon, to recuperate during the evening (Fig. 8).

4. Discussion

$S. densiflora$ is a salt marsh plant species able to tolerate a wide elevational range across the tidal frame.
This study shows how its high ecophysiological versatility allows it to grow in very contrasted salt marsh habitats, where *S. densiflora* responds differently to seasonal and diurnal variations in environmental conditions. Furthermore, this work helps to understand the *S. densiflora* invasion of European marshes and its behaviour in their higher topographical levels.

Net photosynthetic rates of *S. densiflora* recorded in this study were similar to those registered in previous field works in other *Spartina* species (Dai & Wiegert, 1997; Drake, 1989; Pezeshki & DeLaune, 1991). Both *S. densiflora* populations showed high values of WUE, characteristic of the C4 photosynthetic metabolism of this genus (Knapp, 1993). This physiological characteristic, together with the presence of salt glands (Perazzolo & Pinheiro, 1991), would allow them to colonise sediments with high salinity (Pezeshki, Delaune, & Patrick, 1987).

Chlorophyll fluorescence has been proved to be a valuable tool to screen for plant stress in response to various environmental factors (Lichtenthaler & Rindele, 1988). The ratio $F_{V}/F_P$ has been shown to have a value close to 0.835 in unstressed plants (Björkman & Demmig, 1987) and daily fluctuations in irradiance would be expected to result in dynamic photoinhibition that should decrease this value by 15–20% in plants not subject to any other stress (Fernández-Baco, Figueroa, Luque, & Davy, 1998; Ögren & Rosenkvist, 1992). $F_{V}/F_P$ values lower than 0.65 were recorded in many sampled days in both *S. densiflora* populations, indicating a different sort of photosynthetic stress than irradiation.

High salinity and low oxygen concentration have been identified in salt marsh sediments as the main sorts of stress for vegetation. In this study, the higher values in $A$, $A/C_i$, WUE and $F_{V}/F_P$ recorded at the non-tidal population (DIM), except during summer, agree with previous studies in *S. densiflora* that found better physiological functioning at lower salinities (Nieva, Castellanos, Figueroa, & Gil, 1999) and higher redox potentials (Castillo et al., 2000). The response of leaf net photosynthesis to irradiance helped to explain the effects of abiotic environmental conditions on *S. densiflora* populations in the two studied contrasted habitats. Both populations showed a saturated response of leaf photosynthesis to light as recorded in other salt marsh C4 species (Dai & Wiegert, 1997; De Jong, Drake, & F.J.J. Nieva et al. / Estuarine, Coastal and Shelf Science 57 (2003) 1–10
Pearcy, 1982; Pezeshki et al., 1987). However, a clear reduction of A at higher irradiance was recorded in spring and summer in the tidal population, coinciding with the lowest $\psi$ values (ca. −8 MPa). Thus, this drop in A was probably due to water stress. This interpretation is supported by the lack of reduction in A in a foggy day in August (Fig. 6a), that maintained lower vapour pressure deficit than during sunny days.

On the other hand, radiation level is a limiting resource for the production in the lower parts of $S.\ densiflora$ canopy, since the incident light is greatly restricted within tussocks due to the high tiller density of this clonal plant (Nieva et al., 2001). This study shows how low irradiance within tussocks decreased in higher proportion the potential maximum net photosynthesis rate in the $S.\ densiflora$ non-tidal population during low salinity periods than in this population at higher salinities (Mediterranean summer), and in the tidal population that was exposed to high salinities during the whole study. Thus, high growth rates may be reached by $S.\ densiflora$ populations under low salinity conditions when the irradiance would not be a limiting factor, thanks to very high photosynthesis rates, allowing $S.\ densiflora$ to show fast growth rates during spring and summer and after catastrophic events for their tiller population, such as fire (Nieva & Figueroa, 1997).

These results help to explain the high primary productivity recorded in $S.\ densiflora$ populations located on high marshes that produced larger plants (Nieva et al., 2001) with higher growth rates than low marsh populations. $S.\ densiflora$ populations located at lower elevation in the tidal frame not only have lower photosynthesis rate during summer, when sediment salinity was very high; low $T_{1/2}$ values during summer indicated low energy transmission through the photosynthetic electron transport chain (Bolhär-Nordenkampf & Öquist, 1993). However, the non-tidal population (DIM) suffered reductions in A and $A/C_i$ and the lowest photochemical efficiency ($F_V/F_P$) during summer, when sediment salinity was very high; low $T_{1/2}$ values during summer indicated low energy transmission through the photosynthetic electron transport chain (Bolhär-Nordenkampf et al., 1989). $A$ and $F_V/F_P$ increased together with water availability during autumn, however, climatic conditions such as lower temperatures and lower radiation levels were less appropriate for a C4 species than during summer. The decrease in $F_0$ together with higher values of $T_{1/2}$ recorded at the end of autumn suggests a recovery of the energy transmission rates to the photosystems (Bolhär-Nordenkampf et al., 1991). The maximum values of $A$ and $A/C_i$ in the $S.\ densiflora$ non-tidal population were recorded during spring and the beginning of summer, coinciding with its flowering and allowing the high growth rates registered in high marsh populations just before summer drought. The results suggest that water availability and/or hypersalinity of sediment during Mediterranean summer drought limit carbon fixation in $S.\ densiflora$ non-tidal populations. In tidal populations exposed twice a day to flooding, photosynthesis seems to be more influenced by anoxic conditions than by water availability (Castillo et al., 2000).

High levels of photoinhibition, low net photosynthesis rate and low stomatal conductance were recorded on the coldest and clearest day of the study (24th January; minimum temperature was 5°C and irradiance was ca. 800 μmol m⁻² s⁻¹) in $S.\ densiflora$ tidal population, probably caused by an impairment in the electron transport chain (low $T_{1/2}$ values) (Krause & Weis, 1991). $F_0$ values at this time were not specially high, coinciding...
with previous studies that indicated that $F_0$ is not sensitive to cold stress (Epron, Dreyer, & Breda, 1992; Krause & Weis, 1984). The combined effect of high irradiance and low temperature provoked permanent damages to the photosynthetic apparatus of *S. densiflora*; they were reflected in very low values of $F_v/F_p$ throughout the day. Ottander, Hundal, Andersson, Huner, and Öquist (1993) attributed this physiological response to the combined effect of an increase in the excitation energy of PSII and the inhibition of processes related with its reparation. Our results show how low winter temperatures together with high irradiation provoke important damages in the PSII of *S. densiflora*, which may limit its winter production, as well as its invasion of marshes at higher latitudes in the European continent.

Global Climatic Change, is predicted to involve a temperature rise (3°C for next 50 years) and a decrease in rainfall (20% during last 100 years) in the western Mediterranean area (Peñuelas, 2001). This would affect *S. densiflora* populations differently across the tidal frame. The photosynthetic rate in non-tidal populations at high marshes would be reduced for a longer period throughout the year due to higher water and salt stresses. In contrast, tidal populations in low and middle marshes would increase their carbon gain due to higher mean temperatures and less cloudy days. At the same time, the decrease of fresh water input from rivers and streams to Mediterranean salt marshes would provoke spatial redistributions of plant species, in which the physiological traits of *S. densiflora* would be essential for the evolution of vegetation composition. On the other hand, invasion of European marshes by *S. densiflora* at higher latitudes would be favoured by the increase of temperature during subsequent years. These aspects should be topics of future research projects due to the high competitive ability shown by *S. densiflora* in invaded marshes.

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