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G Naidoo a, Y Naidoo a & P Achar b

a School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa

b Department of Biological and Physical Sciences, Kennesaw State University, Kennesaw, GA, 30144, USA

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Ecophysiological responses of the salt marsh grass *Spartina maritima* to salinity

G Naidoo**, Y Naidoo¹ and P Achar²

¹ School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa
² Department of Biological and Physical Sciences, Kennesaw State University, Kennesaw, GA 30144, USA

* Corresponding author, e-mail: naidoogn@ukzn.ac.za

The effects of salinity on growth and productivity of *Spartina maritima* (Curtis) Fernald were investigated in glasshouse and field experiments in 2008. In the glasshouse study, plants were subjected to 2%, 10%, 20%, 40% and 80% sea water, with tidal simulation, for 10 months. Increase in salinity from 2% to 20% sea water increased biomass accumulation, CO₂ exchange, quantum yield of Photosystem II (PSII), electron transport rate (ETR) through PSII, and intrinsic photochemical efficiency of PSII, while further increases in salinity to 80% resulted in significant decreases in these parameters. Concentrations of proline increased significantly with increase in salinity up to 80% sea water. In the field study, soil physicochemical conditions between streamside and inland sites were compared, and the constraints imposed by any differences on plant ecophysiological responses determined. At the inland site, soil water potential (ψ), electrical conductivity of the soil, total cations, and the concentrations of Na⁺, Ca²⁺, Mg²⁺ and P, were significantly higher than those of the streamside site, while CO₂ exchange, quantum yield of PSII, ETR through PSII, and intrinsic photochemical efficiency of PSII were significantly lower. The results suggest that *S. maritima* grows optimally at a salinity of about 20% sea water, and that higher salinity decreases growth and photosynthetic performance.

**Keywords:** biomass accumulation, chlorophyll fluorescence, ion relations, photosynthesis, proline, water potential

Introduction

The salt marsh grass *Spartina maritima* (Curtis) Fernald (cord grass) extends along the Atlantic seaboard from southern Africa to northern Europe (Mobberley 1956) and, like other species of the genus, forms extensive monotypic stands in coastal marshes and estuaries (Adams and Bate 1995, Lee 2003). This species is an important primary coloniser of intertidal mud flats, and contributes to sediment accretion (Cahoon et al. 1996) and elevation of the marsh surface, thereby facilitating successional development (Castillo et al. 2000). Populations of *S. maritima* in Britain and northern Europe lack vigour, rarely reproduce sexually, and are declining, whereas those in Africa are larger, more robust, and produce viable seeds (Raybould et al. 2000, Baumel et al. 2001). Genetic studies indicated that *S. maritima* possesses an extremely low level of genetic variation and will be sensitive to changes in the littoral environment (Yannic et al. 2004). Moreover, the species is dominant in the lower marsh and is therefore particularly vulnerable to sea level rise (van Wijnen and Bakker 2001), especially in areas where it cannot migrate further inland towards areas of lower salinity. Vulnerability may be worsened where freshwater flow into estuaries is reduced due to abstraction (Adams and Bate 1995).

Along the east coast of southern Africa, *S. maritima* forms extensive monotypic stands, especially in perennially open estuaries. Unlike in Europe, southern African populations of *S. maritima* have increased in extent over the last decade. Other species associated with *S. maritima* include *Sarcocornia perennis* (Mill.) Scott, *Triglochin striata* Ruiz and Pavon, *Limonium scabrum* (Thunb.) Kunze, and *Chenolea diffusa* Thunb. in the upper zone, and the seagrass *Zostera capensis* in the lower zone.

Despite the importance and spread of *S. maritima* in African estuaries, very few studies have been undertaken on this species. A laboratory study showed that stem and leaf elongation decreased at salinities above 35 and did not differ between completely submerged and tidally inundated plants (Adams and Bate 1995). In most South African estuaries, freshwater inflows have decreased as a result of dam construction upstream, resulting in mouth closure and increases in water salinity. Global warming and sea level rise will exacerbate both these problems.

There is little information on the salt tolerance of this species and the effects of increased salinity on growth, productivity and photosynthetic characteristics. The hypothesis that increasing salinity in estuaries would decrease growth and productivity of *S. maritima* was therefore tested in field and laboratory studies in 2008. The specific objectives were to investigate the effects of a range of salinities on biomass accumulation, leaf gas exchange, chlorophyll fluorescence and osmotic adjustment. These measures of performance were then compared with soil physicochemical conditions and leaf level gas exchange under natural conditions.
Materials and methods

Plant material
The Keiskamma Estuary (33°17’ S, 27°29’ E), Eastern Cape, South Africa, has an extensive monotypic stand of *S. maritima*, which exhibits a typical, natural productivity and soil salinity gradient from the streamside to inland. Streamside plants are taller with broader leaves, while those inland are shorter with smaller, narrower leaves. The streamside site is regularly inundated by tides, and the average soil salinity is about 30. The inland site is about 60 m inland, at a slightly higher elevation, with a soil salinity of about 42, and is infrequently influenced by tides.

Terminal rhizomes of *S. maritima* were collected from a streamside site in the Keiskamma Estuary and cultivated in 24 cm diameter × 26 cm tall plastic pots containing a mixture of 2:1 sand and potting soil. All pots were maintained in an air-conditioned glasshouse set at 25 °C (day) and 20 °C (night). Pots were placed in troughs and irrigated from below with 10% sea water. Plants were fertilised biweekly with 10% Hoagland nutrient solution (Hoagland and Arnon 1950). After eight weeks, pots containing uniform, actively growing plants were placed in large troughs (80 cm length, 45 cm width, 30 cm height) and subjected to 2%, 10%, 20%, 40% and 80% sea water for 10 months. Seven replicate pots were allocated to each treatment in a completely randomised design. Salinity treatments were introduced gradually at increments of 10% sea water every two days. High and low tides were generated twice daily by a tidal apparatus.

After 10 months of salinity treatments, the plants were harvested. Roots were washed in the respective treatment solutions to remove soil, and then in iso-osmotic mannitol containing 2.5 mol CaSO₄ to remove ions from the free space. Shoots were quickly rinsed in distilled water to remove secreted salts. Plants were separated into above-ground and below-ground components and fresh mass determined. Subsamples of roots and shoots were removed from each replicate, frozen in liquid nitrogen and freeze-dried for the determination of proline. The remaining material was oven-dried at 70 °C to constant mass and weighed.

CO₂ exchange
After 10 months of salinity treatments, photosynthesis was measured with a portable gas exchange system (Li-6400, LI-COR, Lincoln, Nebraska). For each replicate, three measurements were taken at 1 000 μmol m⁻² s⁻¹, 350 μl l⁻¹ CO₂, and 30 °C between 10:00 and 12:00. All measurements were taken on the youngest, fully mature leaves that were exposed to full sunlight.

Chlorophyll fluorescence
Chlorophyll fluorescence was determined with a field-portable, pulse amplitude modulated fluorometer (PAM-2100, Walz, Effeltrich, Germany) using the methodology described by Naidoo (2010). All measurements were taken on the lamina, midway between the base and the tip of mature leaves. Quantum yield of Photosystem II (PSII) electron transport (ΔF/Φ) was calculated as (Fv’/Fm’) × 0.84 × PPFD × ΔF/Φ assuming that 84% of incident light is absorbed by the leaves and that the photons are equally distributed between PSII and PSI (Schreiber et al. 1995). Measurements of chlorophyll fluorescence were taken at saturating light (>800 μmol m⁻² s⁻¹) on the same days and on similar leaves on which gas exchange measurements were made. Five measurements of quantum yield and ETR were taken per replicate. The intrinsic efficiency of light energy conversion of PSII, which is expressed as the ratio of variable to maximal fluorescence (Fv/Fm), was measured after 30 min dark adaptation with a dark leaf clip (Walz, Effeltrich, Germany). One Fv/Fm measurement was taken per replicate per treatment.

Proline
At harvest, subsamples of roots and shoots were excised from each replicate, frozen in liquid nitrogen and freeze-dried. Proline, a compatible organic solute which accumulates in response to salt and drought stress, was extracted from freeze-dried, milled samples with 3 ml of 3% sulphosalicylic acid and determined according to the procedure of Bates et al. (1973). One millilitre of the extract was added to a long screw-cap test tube, together with 1 ml acid ninhydrin and 1 ml glacial acetic acid; the tubes were heated in a water bath at 100 °C for 1 h and the reaction was terminated by transferring the tubes to an ice bath. Thereafter, 4 ml toluene were added and the contents vortexed for 15 s. The chromophore containing the toluene was aspirated from the aqueous phase and allowed to warm to room temperature. The absorbance was determined at 520 nm using toluene as a blank in a double-beam spectrophotometer.

Root distribution
Five PVC pipes (10 cm diameter × 100 cm depth) were used to obtain soil cores from each of the streamside and inland sites along a natural productivity and soil salinity gradient in the Keiskamma Estuary. The cores were extruded intact and divided into 10 cm sections. Living roots and rhizomes were removed from each section and fresh and dry mass determined as described previously.

Soil analyses
Soils from each of the streamside and inland sites were sampled during one neap and two spring tides. Samples were obtained from the centre of well-established streamside and inland sites at a depth of 10–20 cm and processed using the methodology described by Naidoo (2006). Soils were air-dried, crushed with a wooden mallet, passed through a 2 mm sieve and analysed for inorganic ions using the procedures described by Hunter (1975) and Farina (1981). Exchangeable Na⁺ was extracted with 1 M ammonium acetate, Ca²⁺ and Mg²⁺ by 1 M KCl, and K⁺, P, Zn²⁺, Cu²⁺ and Mg²⁺ by the Ambic-2 extracting solution (Hunter 1975). Na⁺, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, Cu²⁺ and Mn²⁺ were determined by atomic absorption (Varian Spectra AA-10, Mulgrave, Australia). P was determined by the
molybdenum blue procedure (Hunter 1975) in conjunction with a spectrophotometer (Beckman DU 640, Fullerton, USA). Saturation extracts of soils were analysed for electrical conductivity using an electrical conductivity meter. Soil pH was determined on air-dried samples using a 1:2.5 soil to 1 M KCl ratio (Hunter 1975). The suspension was stirred for 5 min and allowed to stand for 30 min. The pH was measured with a glass combination electrode while stirring. Bulk density was determined using a plastic cylinder to take a sample of known volume that was then oven-dried at 105 °C and dry mass determined.

**Soil water potential (ψ)**

Soils from the streamside and inland sites were sampled during one neap and two spring tides at a depth of 10 to 20 cm. Soil ψ was determined with a WP4 Dewpoint Potential Meter (Decagon Devices Inc., Pullman, Washington). Five measurements were taken in each of the streamside and inland sites on each of the three measurement days.

**Photosynthetic characteristics of field plants**

Carbon dioxide exchange measurements on streamside and inland plants were taken on three days, including one neap and two spring tides. On each of the three measurement days, 20 measurements of carbon dioxide exchange were taken on streamside and inland plants as described previously.

**Data analysis**

Data were subjected to unpaired, pooled-variance t-tests or to one-way analysis of variance (ANOVA), and to Tukey-Kramer multiple comparisons test, using GraphPad Instat, Version 3. The normality assumption was tested with the Kolmogorov-Smirnov test and the equal variance assumption with the method of Bartlett (Snedecor and Cochran 1989). For heterogeneous variances, continuous data were transformed using logarithms. Multiple measurements of carbon dioxide exchange, chlorophyll fluorescence and soil analyses, taken on each replicate on different days, were pooled by replicate.

**Results**

**Biomass accumulation**

Total dry biomass accumulation was 680% higher in the 20% sea water treatment compared to that at 2%. At 80% sea water, total dry biomass accumulation was 71% lower than that at 20% sea water (Figure 1). Trends in above- and below-ground biomass were similar to those for total biomass. Allocation of resources to below ground increased from 18% at the lowest salinity to 30% at 20% sea water, and thereafter decreased slightly at higher salinities. Although no measurements were taken, plant height, number of culms and leaf size were higher at 20% sea water than at 40% and 80%.

**Photosynthetic characteristics**

Leaf CO₂ exchange was 114% higher where the substrate salinity was 20% compared to 2% sea water. In the 80% sea water treatment, CO₂ exchange was lower than that in the 20% treatment by 52% (Figure 2). Trends in leaf conductance and transpiration closely followed those for CO₂ exchange, while internal CO₂ concentration, Cᵢ, was lower in 40% sea water compared to those in 2%, 10% and 20%. In the 80% sea water treatment, Cᵢ was higher than that in 40% sea water (Figure 2).

Quantum yield of PS II electron transport and ETR through PS II were higher at 20% than at 2% sea water and subsequently declined as salinity increased to 80% (Figure 3). At all salinities, intrinsic PS II efficiency (Fᵥ/Fₘ) was higher in the morning and decreased at midday. Midday depression in Fᵥ/Fₘ was followed by complete recovery by the next morning (Table 1).
Proline
Concentrations of proline were at least three times higher in shoots than in roots, and increased significantly with increase in salinity from 2% to 80% sea water. In the 80% sea water treatment, proline concentrations in shoots and roots were significantly higher than those in 2% by 523% and 746%, respectively (Figure 4).

Soils
The concentration of total soil cations and electrical conductivity of the soil saturation extract were higher at the inland than the streamside site by 23% and 37%, respectively. At the inland site soil concentrations of Na⁺, Ca²⁺, Mg²⁺ and P were higher than those at the streamside site by 47%, 48%, 51% and 48% respectively. There were no differences in bulk density, soil pH and concentrations of Zn²⁺, Mn²⁺ and Cu²⁺ between sites. Soil ψ was −3.08 MPa at the streamside site and −4.32 MPa at the inland site (Table 2).

Root distribution of field plants
The bulk of the root biomass was located close to the soil surface, with streamside plants generally producing more roots at all depths than inland plants (Figure 5). Although no measurements were taken, roots of streamside plants were larger in diameter with a greater cross-sectional air space volume than those of inland plants.

CO₂ exchange of field plants
In streamside plants, mean CO₂ exchange was 11.64 µmol m⁻² s⁻¹, while at the inland site this was lower by 35%. At the inland site, leaf conductance was consistently lower than those in streamside plants, suggesting that light-saturated photosynthesis was conductance limited. Transpiration was 20% lower in inland plants, while Ci was 134% higher (Figure 6).

Discussion
Significant growth stimulation at low to moderate salinity and inhibition at higher salinity (Figure 1) are typical for most monocotyledonous halophytes (Munns 1993, Nieva et al. 1999, Naidoo and Kift 2006). Optimal growth for S. maritima was at 20% sea water, which is similar to those for S. alterniflora (Vasquez et al. 2006), S. anglica (van Diggelen et al. 1986) and S. densiflora (Nieva et al. 2003). Greater allocation of plant resources to below-ground components between 2% and 20% sea water was probably to meet the higher demand for water and nutrients with increasing salinity (Boogaard et al. 1996). At salinities above 20% sea water, disruption of nutrient and water uptake and cause ion imbalance in the leaves, leading to reduced growth (Munns 1993, Yeo 1998). The decrease in below-ground biomass at salinities above 20% sea water would probably reduce...
root surface area and subsequently salt absorption by roots, as well as the volume of the apoplastic pathway that directs salt transport to the xylem. Progressive suppression of whole plant growth at salinities above 20% sea water in glasshouse experiments, as well as in the inland site in the field, was associated with decreases in plant height, number of culms, length and width of leaves and leaf rolling, as reported for other halophytic grasses (Naidoo et al. 2008). Despite growth reduction at 40% and 80% sea water, plants appeared healthy, with no plant mortality, even at 80% sea water for 10 months. The C4 metabolism (Knapp 1993), together with the presence of salt glands (Perazzolo and Pinheiro 1991), probably contributes to the high salt tolerance of *S. maritima*.

The CO2 exchange and chlorophyll fluorescence results (Figures 2 and 3) supported the biomass data that growth was stimulated between 2% and 20% sea water. Reduced CO2 exchange at 40% and 80% sea water in the glasshouse study (Figure 2), and at a salinity of 42 in field plants (Figure 6), is probably a consequence of Na+ build up in the leaves, impaired nutrient acquisition, reduced hydraulic conductivity
and reduced water transport to the leaves (Munns 1993, Yeo 1998, Hasegawa et al. 2000). In Spain, decreased CO₂ exchange in *S. densiflora* during the dry season was attributed to decreased water availability and/or hypersalinity (Castillo et al. 2000).

Parallel decreases in CO₂ exchange and leaf conductance at high salinity suggest that ribulose-1,5-bisphosphate carboxylase/oxygenase generation and ATP synthesis would be impaired (Lawlor and Cornic 2002). Under these conditions, dark respiration rates would probably increase relative to assimilation (Lopez-Hoffman et al. 2007) to meet the costs of intracellular ion compartmentation and salt secretion, contributing to further decreases in CO₂ exchange. In the 40% and 80% sea water treatments in glasshouse experiments, and in inland field plants, the smaller, near vertical, rolled leaves probably minimised high leaf to air vapour pressure deficit, decreased transpiration and salt loading, and contributed to more conservative water use (Bjorkmann and Demming-Adams 1995).

Rates of CO₂ exchange of field plants were comparable to those under glasshouse conditions. In streamside plants, rates of CO₂ exchange (Figure 6) were higher than those inland, probably because of lower salinities and regular tidal flushing that contributed to a more oxidised rhizosphere. In *S. densiflora*, CO₂ exchange in streamside plants was reported to be influenced more by lower salinities (Nieva et al. 1999) and higher soil redox conditions associated with tidal flushing than water availability (Castillo et al. 2000).

A close relationship between salinity tolerance, photosynthesis and growth was reported for *S. townsendii* on the SW coast of Europe (Nieva et al. 1999), and for *S. maritima*, *S. patens* and *S. densiflora* in Louisiana Gulf Coast marshes (Pezeshki and DeLaune 1997).

Decreases in the efficiency of open PSII reaction centres in the light, ETR through PSII (Figure 3), and photochemical efficiency of PSII in dark-adapted leaves (Table 1), support the gas exchange data that CO₂ exchange was reduced at high salinities. At high salinities, chloroplast stromal volume decreases, while reactive O₂ species increase, adversely affecting cell metabolism, photosynthetic capacity and growth (Borsani et al. 2001). Photoinhibition, the light-dependent reduction of the intrinsic quantum yield of PSII and a loss of photosynthetic activity (Osmond 1994), was exacerbated at high salinities. Reduction in photosynthetic efficiency at midday at all salinities, compared to morning values (Table 1), suggested that photoinhibition occurred despite near vertical leaf orientation and leaf rolling. Reduced photosynthetic efficiency at midday has been reported for many species including *S. densiflora* (Nieva et al. 2003). This type of photoinhibition is dynamic, gradual and reversible. Recovery in midday reductions in photosynthetic efficiency during the overnight dark period suggested that photoinhibition was due to down-regulation of the photosynthetic apparatus, rather than to photodamage (Christian 2005).
Accumulation of the imino acid proline in roots and shoots of *S. maritima* in response to salinity (Figure 4) has been demonstrated in many species, including *S. alterniflora* and *S. patens* (Cavaleri 1983, Naidoo et al. 1992) and *S. anglica* (van Diggelen et al. 1986). In all these studies, root levels of proline were low compared to that in shoots, but both increased with a salinity increase. Proline accumulates in the cytoplasm and organelles to balance the low osmotic potential in the vacuole (Binzel et al. 1988, Hasegawa et al. 2000). Proline also preserves protein structure and activity and reduces enzyme denaturation by inactivating hydroxyl radicals and other reactive chemical species (Smirnoff and Cumbes 1989, Saradhi et al. 1995). It is not known whether *S. maritima* also accumulates the osmoregulatory solute, glycinebetaine, like *S. alterniflora* (Cavaleri 1983), or other quaternary ammonium compounds, like *S. anglica* (van Diggelen et al. 1986).

In the field there were distinct differences in soil physico-chemical characteristics between streamside and inland sites (Table 2). At the streamside site soil ψ was close to that of sea water (~2.5 MPa) because of twice daily tidal flushing. Higher evapotranspiration and infrequent tidal influence at the inland site contributed to higher electrical conductivity, higher total cations, higher inorganic ion concentrations, and a lower soil ψ. Plants at the inland site experience greater salt and water stress, as indicated by lower soil ψ. At the inland site the smaller, thicker, near-vertically orientated, rolled leaves indicate a low capacity for water transport, reduced turgor and cell expansion, and ultimately reduced growth.

Similar to other grasses, the bulk of the below-ground biomass was within 10 cm of the soil surface (Figure 5). Greater production of roots and rhizomes possessing larger diameters and greater cross-sectional air space volume suggests that oxygen diffusion rates were probably greater in streamside than in inland plants. Unfortunately, porosity measurements were not undertaken in this study. Others have shown that both *S. maritima* and *S. densiflora* possess well-developed aerenchyma in roots and rhizomes under oxidised conditions (Castillo et al. 2000).

This study has demonstrated that *S. maritima* is a highly salt-tolerant species that can tolerate salinities up to 80% seawater. Similar to most other monocotyledonous salt marsh halophytes, maximal growth occurred at 20% sea water. Higher salinities (40–80% sea water) reduced biomass accumulation and photosynthetic performance. Similar responses to salinity have been reported for *S. alterniflora* (Vasquez et al. 2006), *S. densiflora* (Nieva et al. 2003) and *S. townsendii* (Nieva et al. 1999). Salinity tolerance in *S. maritima* appears to be achieved through increased biomass accumulation, improved photosynthetic performance and changes in resource allocation patterns at low to moderate salinities, the synthesis of the low molecular weight organic solutes such as proline, and probably efficient salt secretion.

The rapid spread of *S. maritima* in South Africa may be attributed to the favourable hydrological regime that produces salinities and soil redox potentials that favour its establishment. In coastal marshes and estuaries the dominance of *S. maritima* in the lower marsh makes it is particularly vulnerable to global warming, especially in areas where the species cannot migrate upstream to areas of lower salinity. Sea level rise, which is predicted to be about 20 cm by 2050 (IPCC 2001), means that marshes at the lowest elevation would be the first to degenerate (van Wijnen and Bakker 2001). Consequently, sea level rise will probably erode the outer boundary of the lower marsh habitat of *S. maritima*. This conclusion is supported by evidence from England where remnants of extensive *S. maritima* populations are frequently confined to eroded, high-level marshes (Raybould et al. 1991). Several recent studies suggest that the genetic diversity of a population may be important in determining responses to environmental change (Fraser and Bernatchez 2001). Additional research is needed to define these conclusions further.

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