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Ion concentrations in seagrass: A comparison of results from field and controlled-environment studies

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ABSTRACT

Osmoregulation is essential for the survival of seagrasses in marine and hypersaline environments. The aim of this study was to examine ion concentrations of four seagrass species (*Posidonia australis, P. sinuosa, Amphibolis antarctica* and *A. griffithii*) after exposure to salinity changes. Plant fragments were placed in a series of aquaria at marine salinity (35) and, after one week of acclimation, exposed for 7 days to salinities between 20 and 70. Cl^-, Na^+ and total ion concentration increased with salinity in leaf tissue of the four seagrasses species. These results were compared with those of *P. australis* and *A. antarctica* samples collected at three locations at Shark Bay, Western Australia where higher salinities occurred, ranging from 46 to 51. Concentrations of K^+ and Ca^{+2} were higher in seagrass tissues from Shark Bay than in those in aquarium trials. Cl^-, Na^+ and total ions in *P. australis* and *A. antarctica* from Shark Bay were lowest at the highest salinity location. The K^+/Na^+ ratio in the aquarium trials (under ambient conditions) was in the following order: *A. antarctica* = *A. griffithii* > *P. australis* > *P. sinuosa* and Ca^{+2}/Na^+ ratio was: *A. antarctica* = *A. griffithii* > *P. sinuosa* > *P. australis*. This species order indicates a physiological capacity to tolerate variation in salinity. Furthermore, these ratios were higher in the locality with highest salinity in Shark Bay, indicating acclimation and adaptation of ion concentrations to the salinity regime in the environment.

1. Introduction

1.1. Osmoregulation in seagrasses

Elevated environmental salinities reduce water potential, making it increasingly difficult for plants to acquire water and nutrients for the environment (see Touchette, 2007 for a review). The ability of plants to tolerate salt is determinate by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis (Parida and Das, 2005). Seagrasses have adapted to life in the sea by developing several strategies to cope with the physiological stresses imposed by salinity (Touchette, 2007), including maintenance of ion homeostasis in order to tolerate salinity changes.

Osmoregulatory adjustments in seagrasses include synthesis of compatible solutes: carbohydrates and free amino acids (Munns, 2002; Touchette, 2007), proline and sugars (Tyerman et al., 1984; Pulich, 1986; Koch et al., 2007; Sandoval-Gil et al., 2012, 2014; Marín-Guirao et al., 2013) and changes in cell ultrastructure (Verslues et al., 2006; Sandoval-Gil et al., 2012), but much less is known about changes in ion content (Tyerman et al., 1984; Tyerman, 1989; Marín-Guirao et al., 2013; Garrote Moreno et al., 2014a,b) that enable seagrasses to cope with gradual and/or pulsed changes in seawater salinity.

Recent studies in several species of seagrasses have observed almost a doubling in concentrations of Na^+ and Cl^- at the highest salinities tested under hypersaline conditions (Garrote-Moreno et al., 2014a, 2015). Marine plants must be able to balance Na^+ and Cl^- fluxes to maintain osmotic equilibrium while preventing the accumulation of these toxic ions within the cytoplasm (Touchette, 2007). It is likely that seagrasses growing within their optimal salinity range can achieve equilibrium fairly rapidly. However, plants exposed to waters outside their typical salinity distribution, but within their tolerance range, may require additional time (days to weeks) to acclimate (Tyerman et al., 1984). When salinity changes persist or become more intense, however,

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the adverse effects of ionic and osmotic stress on metabolism can compromise the productivity and survival of seagrass species (Hasegawa et al., 2000; Zhu, 2003; Parida and Das, 2005; Touchette, 2007). While Cl^- and Na^+ are sequestered in the cell vacuole (Touchette, 2007) they also contribute to maintenance of these ions in low concentration in the cytoplasm preventing physiological damage in addition to osmotic adjustment (Hajibagheri and Flowers, 1989). Maintenance of adequate levels of K^+ and Ca^{+2} is essential for plant survival in counteracting salinity (Maathuis and Amtmann, 1999). Higher K^+/Na^+ and Ca^{+2}/Na^+ ratios are characteristic of more tolerant salinity species (Maathuis and Amtmann, 1999; Muramatsu et al., 2002; Garrote-Moreno et al., 2014a; 2015).

1.2. Seagrasses in hypersaline environments

The extensive and diverse seagrass communities along Western Australia's coastline may be attributed to the general suitability of the coast, which boasts a variety of habitats and a range of tropical and temperate species available for colonization (Kirkman, 1997; Carruthers et al., 2007). *Posidonia australis* and *P. sinuosa* dominate protected habitats in Western Australia, with some *Amphibolis* and other species in small areas. *Amphibolis antarctica* and *P. australis* are Australian endemic seagrasses, widely distributed across southern Australia and reaching their northern tropical limit near Shark Bay on the western coast (Walker, 1985).

Knowledge of salinity thresholds of seagrass species is crucial to understanding and predicting their capacity to withstand chronic changes in salinity regimes, such as hypersaline discharges from desalination plants, and prevent or reduce the impact that this industry may cause some Australian seagrass species. Species of *Posidonia* and *Amphibolis* usually inhabit sublittoral environments with very stable salinity regimes but in Shark Bay (Fig. 1), they also occur in areas with naturally elevated salinity. It is unusual for increased salinity to be maintained at a constant high value in the shallow sedimentary environments with which seagrasses are associated and in this respect Shark Bay provides an almost unique environment for study. Although there are some variations in surface salinity, bottom salinity remains relatively constant on a seasonal basis (Logan et al., 1974) and on a longer time span, no significant differences in the spatial pattern of salinity distributions were observed by Smith and Atkinson (1983). Shark Bay, Western Australia, consists of two gulfs more than 200 km long; open to the Indian Ocean at their northern ends. The restriction on seawater circulation imposed by broad, shallow shoals across the northern ends of the gulfs combined with the high rate of evaporation in the arid, subtropical climate, results in a gradient in salinity from north to south. Salinity reaches almost double that of seawater salinity at the southern ends of the gulfs, where evaporation exceeds precipitation by a factor of 10, and where there is a persistent gradient in salinity which increases from oceanic (35) to almost twice that of seawater (70) in the southern sections of the bay (Logan et al., 1974; Smith and Atkinson, 1983; Hetzel et al., 2015). Shark Bay has a remarkable and diverse seagrass flora, with 12 species recorded, including some species from the temperate genera, *Posidonia* and *Amphibolis* (Walker et al., 1988). The occurrence of seagrass species is influenced by the prevailing salinity, allowing individual species to be examined along the gradient of salinity at the upper limit of their tolerance to salinity. Extensive seagrass meadows cover much of the vast area of Shark Bay (surface area 13,000 km^2), forming the biggest seagrass banks in the world. They are dominated by *A. antarctica,* which covers 3700 km^2, with smaller areas of *P. australis* (200 km^2) (Walker, 1985). Earlier studies showed a positive correlation in distribution, biomass and in situ productivity of *A. antarctica* with increasing salinity, up to an optimum growth rate at 42, and then decreasing as the salinity increased, as well as at lower oceanic concentrations (Walker, 1985; Walker et al., 1988).

1.3. This study – aims

The aim of this study was to examine changes in concentrations and ratios of Na^+, Ca^{+2}, K^+ and Cl^- in leaf tissue of four SW Australian seagrass species (*P. australis* Hook. *f.*, *P. sinuosa* Cambridge and Kuo, *A. antarctica* (Labill.) Sonder *et* Ascherson and *A. griffithii* den Hartog) over a range of salinities (20–70) in aquarium conditions. These ion concentrations were then compared for two of these species, *P. australis* and *A. antarctica* from three locations in Shark Bay with naturally elevated salinity (Monkey Mia (46), Denham (46.35) and Nanga (51), where the occurrence of seagrass species is influenced by the prevailing salinity.

2. Materials and methods

2.1. Plant sampling and experimental mesocosmos design

Fragments of rhizome with intact connected shoots and roots were collected from a shallow bed at 1–1.5 m depth located at Woodman Point (33° S, 112°) near Perth, in October–November 2010. Plants were brought to The University of Western Australia (Perth, Australia) in coolers from the sampling site and transplanted within 4 h. The mesocosms consisted of six 200 L aquaria in a constant temperature facility at UWA with constant temperature, kept at 22° C. Increased salinity treatments were produced by adding marine salt to seawater and the lower salinity was obtained by diluting seawater with distilled water. Salinity levels were maintained within ±1.5 throughout the experiment (here salinity is measured and reported according to the practical salinity scale). The light regime was adjusted to 12:12 h (light:dark) with additional overhead fluorescent lights. Independent air pumps were installed to maintain proper aeration in each aquarium. The parameters of illumination (irradiance and photoperiod), water temperature and water refill were kept constant during all experimental process, so salinity was the only introduced variable after the acclimation period. The species tested have similar thermal optima in the warm temperate range. They inhabit areas where mean water temperatures have a rather restricted annual range (Cambridge et al., 1991; Walker and Cambridge, 1995). After one week acclimation period, plant fragments were exposed for 7 days to 20, 35 (ambient salinity, control treatment), 40, 50, 60 and 70 for *P. australis* and *P. sinuosa*; and 20, 35 (ambient salinity, control treatment), 45, 60 and 70 for *A. antarctica.* Due to the scarcity of *A. griffithii* at the sampling sites, the salinity treatments for this species were restricted to 20, 35 (control treatment) and 70.

P. australis was collected at three locations at Shark Bay some 830 km north of Perth: Monkey Mia (46), Denham (47) and Nanga (51) at approximately 2 m depth. *A. antarctica* was only found at two of the three stations, Monkey Mia and Nanga (Fig. 1). Water samples were measured at each site for analysis of background salinity with a refractometer. The samples were collected by snorkling in the morning and then kept in coolers until processing within a few hours at Denham, using the same techniques as the aquarium samples (described in 2.2).

2.2. Plant response measurements

Total surface area (cm^2 $shoot^{-1}$) and number of leaves per shoot was measured on ten replicates for *P. australis* and *P. sinuosa.*