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The seed banks of two temporarily open/closed estuaries in South Africa

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ABSTRACT

The seed banks of two temporarily open/closed estuaries in South Africa were quantified in this study. Charophyte öospores represented almost 72% of the sexual propagules in the sediment with a mean öospore density of 31,306 öospores m^{-2}. This was followed by the seeds of the intertidal salt marsh plant *Sarcocornia perennis* (18%) (7929 seed m^{-2}) and the submerged angiosperm *Ruppia cirrhosa* (7%) (2852 seeds m^{-2}). The remaining 3% was made up of a mixture of species such as *Salicornia meyeriana*, *Sporobolus virginicus*, *Stukenia pectinata*, *Bolboschoenus maritimus* and terrestrial species. Although seed density did not differ significantly with depth, seeds still occurred at 20 cm depth providing a regeneration source in the event of sediment disturbance. Three salinity (0, 17 and 35 PSU) and moisture treatments (exposed, waterlogged and submerged) were applied to collected sediment to determine how fast species would germinate. *S. perennis* germinated after 3 d to a maximum of 82%. Submerged species began to germinate only after 18 d (*Chara vulgaris* and *R. cirrhosa*) and had low germination percentages of between 11 and 15% after 91 d. Results from this study indicate that in the event of unpredictable disturbance events such as water level fluctuations, large sediment seed reserves would ensure habitat persistence.

1. Introduction

Temporarily open/closed estuaries can show large variations in environmental conditions, mainly salinity and water level, driven by freshwater inflow (Van Niekerk et al., 2008). These variations can be so extreme that whole plant communities may be lost after each disturbance event. Salt marsh for example, is permanently lost after 3 months submergence (Riddin and Adams, 2008), whereas submerged macrophytes are lost within days due to exposure and desiccation when the mouth of the estuary breaches (Adams and Bate, 1994). These changes take place rapidly and at unpredictable frequencies. Persistence of macrophyte habitats depends on the rate and extent of recovery. Recovery can take place through either vegetative/asexual growth (e.g. rhizomes and tubers that exist in the sediment) or through viable seed/sexual reserves in the sediment. Studies have shown that as habitat disturbance increases, growth from seed reserves becomes more important (Casanova and Brock, 1996; Combroux and Bornette, 2004). Although most seed occurs within the top 5 cm of the sediment, seed reserves with depth can be a source of propagules in areas where disturbance occurs (De Winton et al., 2000; Dugdale et al., 2001).

Seed banks have been widely quantified in freshwater marshes and wetlands (Harwell and Havens, 2003; Shili et al., 2007) and in coastal salt marshes (Grillas et al., 1993; Wolters and Bakker, 2002). However limited quantification of seed reserves in temporarily open/closed estuaries have been reported internationally (Gesti et al., 2005; Shaw et al., 2008). No studies of this nature have been done on temporarily open/closed estuaries in South Africa. A few studies have focussed on the germination requirements of estuarine plants (Naidoo and Naicker, 1992; Naidoo and Kift, 2006; Shaw et al., 2008). Germination rates also give an indication of how quickly habitats respond when optimum conditions occur. The main objective of this study was therefore to quantify and describe the seed banks of two temporarily open/closed estuaries. Germination rates of key estuarine species under three salinity and three water level treatments were also assessed. It was hypothesised that seed numbers will be comparable to other unpredictable habitats that show large environmental variations. This study forms part of a larger multidisciplinary study on the functioning of temporarily open/closed estuaries in South Africa. Data will provide an understanding of the dynamics of macrophytes in these highly variable and unpredictable ecosystems.

2. Materials and methods

2.1. Site description

The East and West Kleinemonde estuaries are situated 15 km north-east of Port Alfred in the Eastern Cape of South Africa (33°32′S, 27°03′E) and are about 400 m apart. At times in the past

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the two estuaries shared the same mouth. The East Kleinemonde Estuary has a smaller catchment area (46.3 km^2) than the West Kleinemonde (93.7 km^2) (Badenhorst, 1988) and the mouth opens and closes rapidly in response to freshwater inflow. The surface area of the West Kleinemonde is approximately 80 ha when full, in comparison to 35.7 ha in the East Kleinemonde Estuary. Both estuaries can remain closed for extended periods of time (e.g. 2 years) due to the formation of a sand bar at the mouth. During the study period the mouth of the East Kleinemonde Estuary opened more frequently than that of the West Kleinemonde. The mouth in both estuaries may remain open for a few days to a few weeks. Intertidal salt marsh in both estuaries is characterised by *Sarcocornia perennis* (Mill.) A.J. Scott., *Sporobolus virginicus* (L.) Kunth and *Salicornia meyeriana* (Moss). When the mouths of both estuaries are closed, indundated salt marsh is replaced by submerged macrophytes after 2 months (Riddin and Adams, 2008). Submerged species are characterised by Charophytes *Chara vulgaris* L. and *Lamprothamnium papulosum* (Wallr.) J. Gr. and angiosperms *Ruppia cirrhosa* (Pentagna) Grande and *Stukenia pectinata* (L) Börner (*Potamogeton pectinatus*) depending on the salinity at the time. Water level fluctuations of up to 1 m occur at the sample sites for both estuaries. In a previous study vegetation change in response to water level fluctuations was investigated along three permanent transects at three different sites in the East Kleinemonde Estuary (Riddin and Adams, 2008). Percentage cover abundance of extant vegetation was measured every 5 m within duplicate 1 m^2 quadrats over the length of each transect. Data from this study, as well as data based on visual observations from the West Kleinemonde were used to relate vegetation cover to sediment seed density in this study.

2.2. Seed density

Three sites were selected in both estuaries in areas where the vegetation changes in response to water level fluctuations. Sites in both estuaries were sampled in March, May 2006 and in February 2007. In addition to this the West Kleinemonde Estuary was sampled in August 2006 when the mouth of the estuary opened and seed sampling sites were exposed and in the East Kleinemonde Estuary in November 2006 when water level was low. At each site, 45 sediment cores were randomly collected within a 10 m × 10 m plot (4 cm diameter and 5 cm deep). To account for the spatial heterogeneity of seed distribution, samples for each site were aggregated into separate buckets and then sub sampled. The surface area of the aggregate sample for each site represented 0.0565 m^2. The buckets were closed and stored at 4 °C until analysis.

The seed banks were quantified using the direct counting method. This method is less commonly used than the indirect or seedling emergence method. However since little information exists on the effects of storage conditions and germination requirements of estuarine plants in this study, the direct counting method was considered to give a more accurate estimate of viable seed density. From the collected aggregate sample for each site, three sub samples of 100 mL each were analysed for seed numbers. Each sub sample was wet sieved with tap water through a 250 μm sieve. This sieve size represents the smallest propagule size, namely the öospore of Charophytes (hereafter included in the terms "seed" and "seed banks"). The concentrated seed sample was then vacuum filtered onto filter paper through a Buchner funnel to remove excess water. The filter paper containing the seed material was left to air dry at room temperature for a minimum of 3–4 d. Once dried, the concentrated sample was analysed under a dissecting microscope. Seed numbers were extrapolated to express seed density per m^2 for the combined sites. The percentage of seeds represented by the dominant estuarine species was calculated. Viable seeds and öospores were identified as those having an intact seed coat, turgid condition (by applying a light pressure to the propagules) and healthy starch reserves when squeezed (Casanova and Brock, 1990).

The distribution of seeds with depth was measured from sediment cores taken in May 2006 at 0–5, 5–10 and 10–20 cm depths in the East Kleinemonde Estuary. This was a preliminary investigation on depth distribution and therefore sampling only took place at the one estuary. At three sites in the estuary 45 core samples were collected for each of the three depths and aggregated. At Site 3 (EK3) sediment at 10–20 cm could not be collected due to hard bedrock. From each aggregate sample 3 sub samples were analysed. The procedure outlined in the previous paragraph was used to determine seed density and percentage representation of the dominant estuarine species.

2.3. Germination rates

The seedling emergence method was used to measure germination rates for the dominant estuarine species using three salinity treatments (0, 17 and 35 PSU) and three water level treatments (E = exposed, W = waterlogged and S = submerged). Salinity and hydrological conditions are considered the two main environmental variables influencing germination in estuarine plants (Kahn and Gul, 2006). Samples from March 2006 were used to assess germination rates for seeds from the East and West Kleinemonde estuaries. Sediment from three different sites in each estuary was exposed to the different treatments. From the aggregated sample per site, 50 mL of sediment was spread over a 4 cm layer mixture of potting soil and river sand in 12 cm diameter pots. Three replicate samples were used from each site. The data were pooled for the sediment from the two estuaries as percentage germination was expressed per species. This mixture was used since pure potting soil can result in gas formations and algal blooms in submerged samples (Boedeltjie et al., 2002). Seawater was diluted with fresh tap water to obtain 17 PSU. For the submerged treatments, pots were placed in plastic drums and a water level of 5 cm was maintained over the sediment surface by topping up as and when needed. Waterlogged treatment pots were placed in plastic drums and stood in water so that the surface of the sediment always remained moist. Exposed samples were watered when dry with fresh tap water. A control pot was used to determine the presence of seed in the potting soil and seed dispersal in the greenhouse. The experiment ran for a period of 91 d, adequate time for both submerged and intertidal salt marsh species to germinate. Glasshouse temperatures ranged from 2 to 36 °C and photoperiod ranged from 12:12 (L/D) to 10:14 (L/D) at the end of the study (South African Weather Bureau). Emergence of seedlings was assessed initially every 3 d for the first 2 weeks and thereafter at weekly intervals until the end of the experiment. Seedlings were counted and removed after identification so as to remove any harmful allelopathic or competitive interactions. Emergence was defined as the development of a germinated seedling to a stage where it could be detected by eye (De Winton et al., 2000). After completion of the experiment, sediment from the pots was sieved through a 250 μm sieve to extract any remaining seed so that percentage germination could be calculated. Germination rates were expressed as the accumulative number of seedlings (germination %) germinating over the trial period.

2.4. Data analysis

A one-way ANOVA was used to determine the significant difference among the mean seed density in both East and West Kleinemonde estuaries, as well as with depth. When significant