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The Ability of *Ruppia polycarpa* to Regenerate from Seed Depends on Seasonal Porewater Salinity Dynamics and Declining Winter Rainfall Could Delay Recruitment

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Abstract

For many plants, regeneration from seed is vital for population recovery. Climate change is modifying the global hydrological cycle and a primary factor controlling germination of marine plants: salinity. How altered salinity regimes, and especially altered porewater salinity regimes, will regulate early life history stages of estuarine seagrasses is poorly understood. Here, we quantified the porewater salinity dynamics in two ephemeral estuaries that are dominated by the cosmopolitan genus *Ruppia*. Seedbank, germlings (germinated seeds) and seedlings were found in salinities ranging from 5 to 110 over an annual cycle. To understand the germination ecology of the dominant species, *Ruppia polycarpa*, seeds were exposed to treatments simulating current salinity regimes and those predicted under climate change. Seeds underwent a Dormancy treatment (15, 60, 150) followed by a Germination treatment (10, 20, 80). Generally, early life history stages were positively affected by hypersaline dormancy conditions if the subsequent Germination salinity was ≤ 20. Germination success was significantly higher for seeds transferred to 10 (65%) compared to 20 (49%) whilst no seeds germinated in 80 highlighting the risk of lower germination as estuaries become drier and more hypersaline with declining winter rainfall. However, germlings were found in situ in salinities ≥ 80 suggesting aspects of the salinity dynamics, not captured by our experimental conditions, may broaden tolerances. Dormant seeds were continuously present in situ and seedlings were observed throughout the whole of the growing season. These results are indicative of bet-hedging strategies. Future research should explore the capacity of these strategies to afford resilience to *R. polycarpa* to salinity variability under climate change.

Keywords Dormancy · Germination · Porewater salinity · Recovery · Bet-hedging

Introduction

Foundation marine plant species improve ecosystem function and provide goods and services which are integral to the livelihood and well-being of humanity (Gaylard et al. 2020; Kendrick et al. 2019; Orth et al. 2006). Management of these species has shifted from a focus on current condition to enhancing ecological resilience so that populations persist, and goods and services are retained in the long term (Collier et al. 2020). To achieve this objective, the important drivers that promote population persistence over time need to be known (Borja et al. 2013). In complex ecological systems, where human modification is extensive and the influence of climate change is amplified, there is considerable uncertainty about the factors that are most important for conferring resilience (Ontoria et al. 2019).

Anthropogenic climate change is altering the climate including critical environmental cues that could impact the ability of organisms to complete their life cycles (Radchuk et al. 2013). Germination is one of the earliest stages in the life cycle of plants and generally requires particular environmental conditions, or combinations of these, for seed dormancy release to prompt germination (Shan et al. 2018). The more specific the requirements of germination, the more vulnerable this stage to the effects of changing conditions associated with climate change (Infante-Izquierdo et al. 2019) For terrestrial plants, understanding of germination

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conditions has enabled predictions regarding the effects of climate change (Mondoni et al. 2012) but understanding to enable these predictions for aquatic plants is limited (York et al. 2017). The germination strategy and variety of germination requirements between and within species will interact to influence the effects of climate change. Species that adopt a generalist germination strategy can germinate occur over a broad range of conditions which is likely to be more beneficial under increasingly unpredictable conditions than species with a specialist strategy that have more specific germination requirements (Barga et al. 2017). Global warming is altering environmental conditions in coastal ecosystems that could affect the timing and success rates of recruitment from seeds (Orth et al. 2000). Salinity is affected by evaporation, rainfall patterns and oceanic exchange and which in turn are also influenced by climate-driven gradual warming and extreme events (Tweedley et al. 2019). Marine and freshwater flowering plants are currently under-represented in studies to understand seed traits and germination strategies which limits the capacity to predict the effects of climate change on recovery dynamics (Kendrick et al. 2022).

Estuaries rank amongst the most productive ecosystems on earth owing to the environmental heterogeneity created by the salinity gradient (McKinney et al. 2006; Veale et al. 2014). Salinity is affected by drivers of global change including temperature, precipitation and sea-level rise (Poloczanska et al. 2007). Between 2011 and 2020, global surface temperatures were 0.88 °C higher than those in 1850–1900 and the regularity of marine heatwaves has doubled since the 1980s (Intergovernmental Panel on Climate Change 2021). Higher temperatures result in greater evaporation, drying and increased propensity towards hypersalinity (Tweedley et al. 2019). Given estuaries are typically shallow and oceanic exchange is limited naturally (Scanes et al. 2020) or by human modification (Cyrus et al. 2011), salinity regimes in these ecosystems may be at greater risk of modification under climate change (Du and Park 2019; Wilson and Dunton 2018). For many plant species in estuarine environments, the salinity gradient governs the life cycle strategy they adopt (Brock 1986). The more variable the habitat salinity regime, the more likely the occurrence of species that adopt an annual life cycle such as *R. polycarpa* and *R. tuberosa*, whereas in stable salinity habitats, *R. megacarpa* tends to dominate and adopts a perennial life cycle (Brock 1983). With the emergence of more severe salinity conditions from climate change, species may die off more frequently increasing the dependence on sexual reproduction and a sediment seedbank for recovery (Statton et al. 2017) and putting long-term persistence at risk (Wilson and Dunton 2018).

Seagrasses are critical habitat-forming species that perform unique ecological and economic roles in estuaries (Beck et al. 2001; Hanington 2014). Worldwide loss of seagrasses has resulted in increased emphasis on understanding the factors that influence their recovery. For seagrasses that rely mainly on germination from the sediment seedbank for meadow regeneration, the environmental conditions required for germination processes represent a major knowledge gap (Kendrick et al. 2022). Generally, for species that produce dormant seeds, low salinity conditions, usually following the wet season, are considered necessary to break physiological dormancy and induce germination (Harrison 1991). This understanding has emerged from field observations and experiments that replicate the range of surface water salinities within a habitat (Carruthers et al. 1997; Kim et al. 2013). However, salinity of the sediment porewater, where dormant seeds reside, can differ from the surface water salinity because of seasonality, sediment properties, tidal inundation and evapotranspiration (Moffett et al. 2012). Thus, quantification of porewater salinity may help to delineate the conditions required to break dormancy and initiate seed germination. Germination requirements may also vary amongst populations. For instance, Koch and Seeliger (1988) found that for *Ruppia maritima*, seeds from a salt marsh characterised by fluctuating salinity germinated across a broader range of salinities than seeds from a habitat with more stable salinities. Thus, porewater salinity conditions during dormancy and germination have the potential to influence germination success and the amount of subsequent recovery but responses to changes in salinity may also differ amongst populations exposed to different local conditions. Experiments exposing different populations of a species to similar environmental conditions are useful to understand whether variation in response has a genetic (adaptation) or phenotypic (acclimation) basis (Connolly et al. 2018; Maxwell et al. 2014).

Species of the genus *Ruppia* occur across all six global bioregions (Short et al. 2007) and are ecologically important, providing food and habitat for fish and waterbirds (Keats and Osher 2007; Tatu et al. 2007). These species tolerate a range of environmental conditions and so usually dominate variable and disturbed coastal ecosystems, with their abundance and distribution within those systems driven mainly by salinity fluctuations (Strazisar et al. 2015). Extreme salinities and extreme variation in salinity have been implicated in the disappearance of *Ruppia* spp. from estuaries in the United States and Australia, due to reduced freshwater inflows and evaporation (Strazisar et al. 2013a; Whipp 2010). In Australia, current observations and predicted trends include more regular and persistent hypersalinity in estuaries (Hallett et al. 2018). These scenarios could affect the ability of the four *Ruppia* spp. that occur in the continent to germinate and recover (Brock 1986), making it a useful case study for examining the impacts of altered salinity regimes on seagrass resilience that might be expected in habitats that seasonally dry out and are common in Mediterranean-climate regions. Whilst considerable work has been done to understand the germination ecology of *Ruppia* spp. (Brock 1982; Koch and Seeliger 1988; Strazisar et al. 2021), variation in salinity tolerances amongst populations suggests the need for system-specific studies to investigate germination cues and inform management.

The aim of this study was to understand the germination ecology of *Ruppia polycarpa* in relation to porewater salinity dynamics. In a field study, we quantified the range of porewater salinities that occurred over the complete life cycle of *R. polycarpa* including in areas that seasonally dry out. We then used this information to experimentally test how different combinations of dormancy-period salinity and germination-period salinity affect seed germination, time to germination and seedling establishment, including salinity conditions that mimicked future, climate change-driven conditions.

Methods

Two seasonally hypersaline estuaries form part of the Vasse Wonnerup Wetland System in southwestern Australia (Fig. 1). The seagrass *R. polycarpa* dominates and co-occurs with *R. megacarpa*. The life cycle of both species is annual with meadows relying on their seedbank for regeneration each winter, when freshwater flows into the system and salinity is generally lower, and dying off completely towards the end of summer when salinity increases with more intense evaporation and the upper reaches partly drying out. Long-term monitoring has generated some understanding of how surface salinities affect the adult standing stock of *Ruppia* spp. (Paice and Chambers 2020) but porewater salinity has not been measured and its effects on early life history stages are unknown. The average salinities are generally higher in the Wonnerup but the range is broader in the Vasse with lower minima and higher maximum salinities, although there is inter-annual variation in these patterns (Table 1). The Vasse estuary is fed by three rivers with varying flows and which may result in more temporal and spatial variation in salinity than in the Wonnerup which is fed by a single river (Department of Water 2010) (Fig. 1). Surge barriers on the exit channels of each estuary can be opened at different times and for varying durations and may also produce differences in salinity (*pers comm* L. Kalnejais).

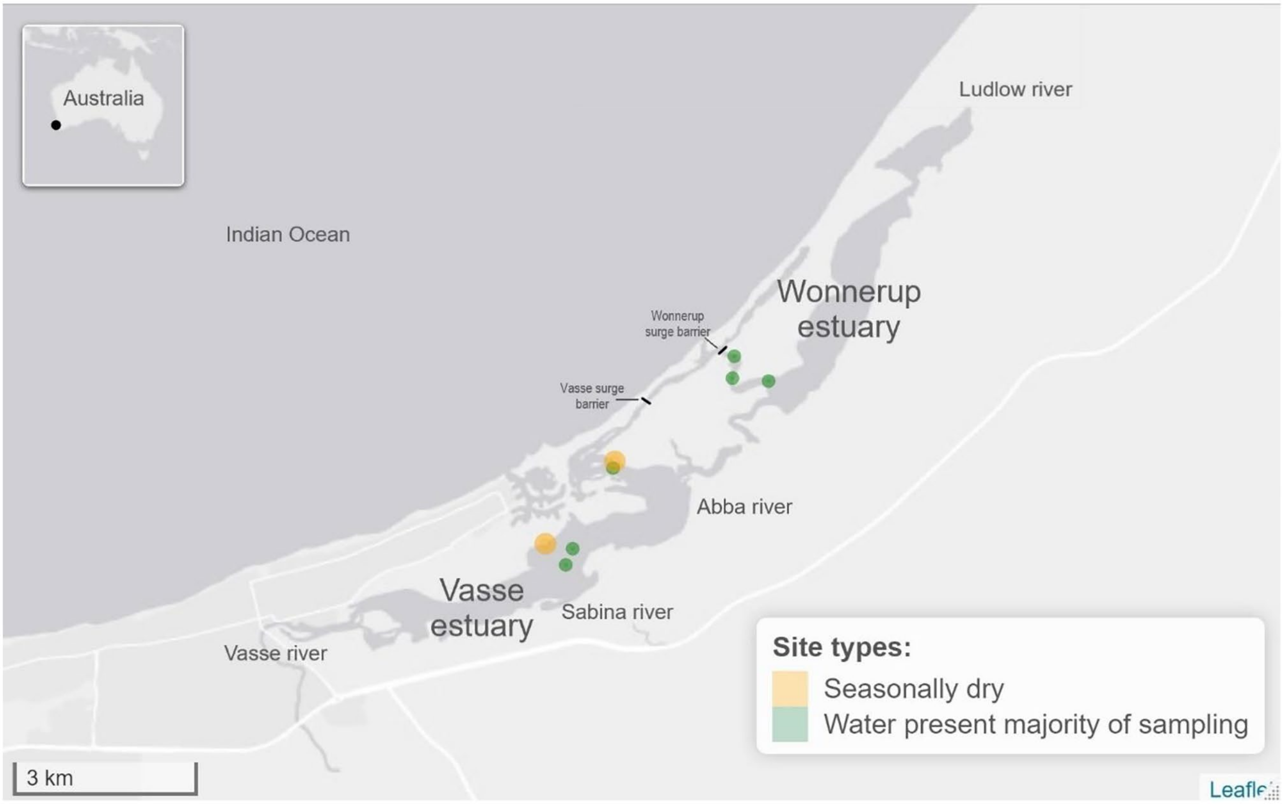

Fig. 1 Vasse and Wonnerup estuaries in southwestern Australia. All sites where sediment cores were collected to assess the depth-distribution of early life cycle stages of *Ruppia* spp. and porewater salinity conditions were upstream of the surge barriers. One core was collected from six sites (green) which retained water from July 2019 to January 2020($n=1$/month). Three cores were collected from three zones in the areas that seasonally dry in June 2019 and January 2020 (yellow) ($n=3$/month)

Table 1 Annual mean (minimum–maximum) salinity. Data retrieved from Water Information Reporting website

Year	Vasse estuary	Wonnerup estuary
2016	6 (0–96)	10 (0.4–48)
2017	10 (0.4–51)	15 (1.0–38)
2018	23 (0.9–97)	14 (0.6–86)
2019	19 (0.8–83)	23 (1.6–83)
2020	17 (0.6–72)	17 (3–66)

Field Sampling (Porewater and Early Life Stages)

Field sampling was conducted to characterise the range of porewater salinities that the sediment seedbank is exposed to during critical life history stages using two approaches. First, porewater salinity changes were characterised at sites where seagrasses completed their full life cycle from early germination to senescence. One sediment core was collected each month from June 2019 to January 2020 from three sites in each of the Vasse and Wonnerup estuaries (Fig. 1). For the second approach, porewater salinity was evaluated at two sites in the Vasse Estuary that dry out seasonally but support seagrass during their inundated period and were likely to display more extreme salinities than the permanently inundated sites. Sampling was conducted at the end and at the start of the dry season (June 2019 and January 2020, respectively), when the seedbank is usually dormant and no adult plants were present. Within each site, three replicate cores were collected from each of three zones which differed in their degree of drying: above the water's edge, at the water's edge and in the shallow inundated. The information from the field sampling was used to inform the treatments for the germination experiment. For both approaches, sediment cores (9.6 cm Ø) were pushed to a depth of 15 cm to collect sediment and porewater. A bung was placed immediately at the bottom of the core, surface water was removed by syphoning and another bung was placed at the top of the core. Cores were secured upright and transported back to Edith Cowan University for processing. Within 48 h of collection, cores were sliced at 5-cm intervals and each slice placed in a separate sealed bag and stored at 4 °C prior to processing. Porewater salinity was measured at three depth intervals (0–5, 5–10 and 10–15 cm) by extracting the porewater with a Wildco™ sediment squeezer with 100 µm mesh and measuring salinity using a conductivity meter. After porewater salinity was measured, each sediment depth interval was placed on a 750-µm sieve which trapped seeds, germlings and seedlings that are > 1 mm in diameter (Brock 1982) and rinsed with seawater. Following Mason (1967), all three life stages were identified to species and then counted. Only seeds within intact seed coats were included in the seedbank density count. Germlings were defined as those that had an emerging cotyledon (> 1 mm but < 10 mm) indicating germination had occurred whilst seedlings were categorised when the leaf was longer than 1 cm and adventitious roots were present, indicative of successful establishment (Gu et al. 2018).

Seed Viability Assessment

Seedbank viability assessments were conducted on seeds of *R. polycarpa* (155) and *R. megacarpa* seeds (77) collected in February 2021. Within a week of collection, seed embryos were separated from their seed coats and placed in 1% tetrazolium chloride solution for 48 h in darkness. Seeds examined under a dissecting scope were considered viable when the embryo stained red (Hendry and Grime 1993).

Seed Collection

Seeds of *R. polycarpa* were collected directly from the reproductive shoots of adult plants from the Vasse and Wonnerup estuaries in November 2019 and kept in water from the collection site during transportation. In the laboratory, seeds from each estuary were stored in separate tanks (28 × 20 × 14 cm) covered with black plastic. Each tank contained seawater diluted with rainwater to a salinity of 15 and maintained at 21 °C, (i.e. the salinity and temperature at the time of collection). The seeds were maintained under these conditions for 9 months until the experiment began, at the time when seed germination is observed in the Vasse and Wonnerup estuaries.

Experimental Design

A mesocosm experiment was designed to test the effects of the estuary of origin (Estuary, levels: Vasse, Wonnerup); porewater salinity when the seed is dormant ('Dormancy', levels: 15, 60, 150); and the porewater salinity when the seed is germinating ('Germination', 10, 20, 80) on the germination of *R. polycarpa* (Fig. 2). The levels within each porewater salinity factor reflected the range of porewater salinities that seeds, germlings and seedlings are currently exposed to in the Vasse and Wonnerup estuaries (see the "Results" section for details) and may be exposed to under a drying climate (Hallett et al. 2018; Hughes 2003). The experiment was conducted in an outdoor mesocosm facility from August to October 2020 and comprised two phases. During phase 1, seeds were subjected to one of the Dormancy conditions for nine days and then, in phase 2, to one of the Germination treatments for 40 days (Fig. 2). For phase 2, a random block design was used with three replicate Blocks set up per estuary ($n = 3$). Each Block was a large (850 L), temperature-controlled water tank and within each, were nine smaller (5 L) tanks. Each smaller tank contained ten seeds and was

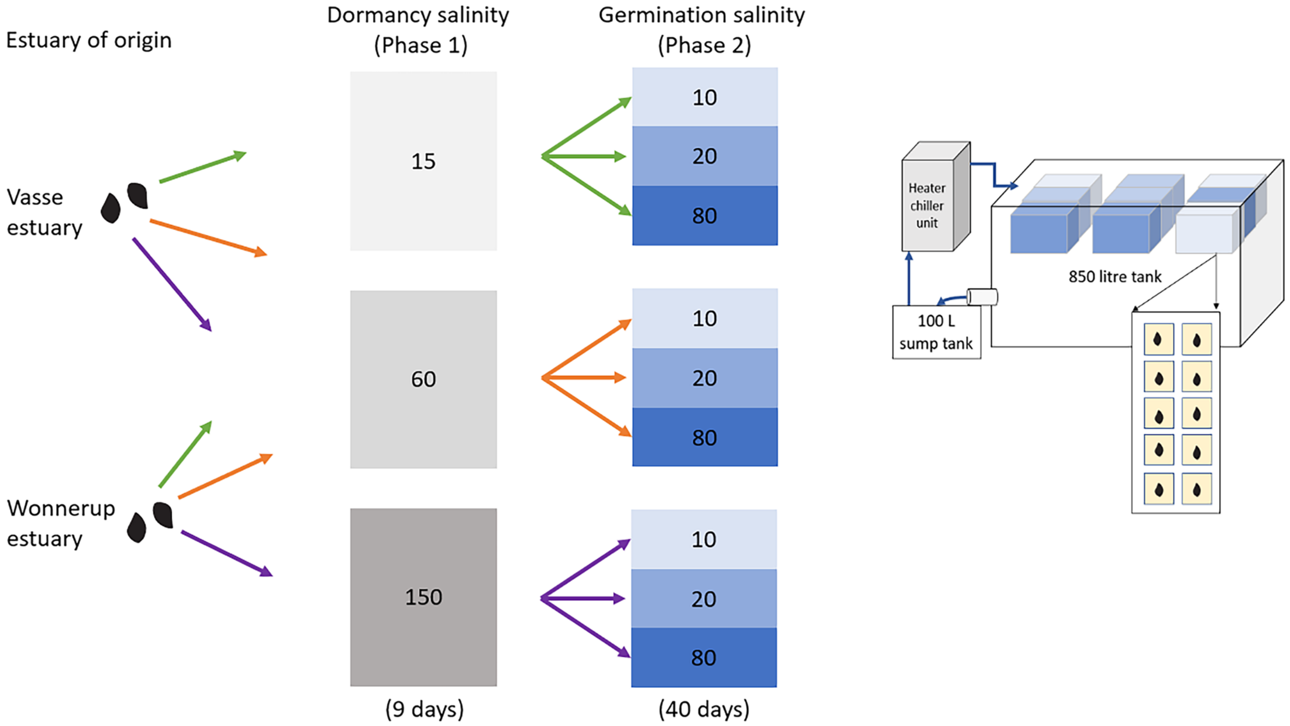

Fig. 2 Experimental design to test the effects of estuary of origin, porewater salinity during dormancy and germination on the germination ecology of *Ruppia polycarpa*

randomly assigned one of the 9 Dormancy × Germination treatment combinations, so that across all 3 Blocks, there were 3 replicates of each combination (Fig. 2).

Treatment salinities were made by mixing freshwater and Red Sea Salt Coral Pro Marine Aquarium Salt™. During the Dormancy phase, seeds were placed into tanks containing the solution (without sediment), in darkness with water temperature initially 21 °C and reduced by 1 °C per day to reach 13.5 °C, the in situ peak germination temperature. No seeds germinated during this phase. For the Germination phase, each small tank within each Block had 10 plastic cells filled with a mixture of washed sand (98%), dried macrophyte wrack (1%) and an inoculant (1%) by weight (~90 g) of sediment from the collection site. One seed was placed in each cell just below the sediment but with the beak still visible so that the emergence of the cotyledon, indicative of germination, could be noted (Koch and Seeliger 1988). Each small tank was filled with 3 L of the Germination salinity solution with around 5 cm of the tank remaining above the water level in the Block tank so that the salinity was maintained. Mean daily sunshine hours ranged from 6 h in August to 8 h in October (www.bom.gov.au). Average in-water midday light intensity was 373 µmol photons m^{-2} s^{-1} measured using a MicroPAR quantum sensor In-situ Marine Optics Pty Ltd, Western Australia.

Salinity and temperature were checked daily with a Thermoscientific multimeter in each tank. Salinity adjustments were made to ensure salinity was ± 0.5 PSU of target salinity. Water levels were maintained via additions of premade salinity treatment solutions to reduce salinity variations due to evaporation. Temperature was maintained using heater-chiller units (Teco TK2000). One HOBO data logger (UA-001–64) was placed in the central small tank in each Block to measure temperature at hourly intervals. Mean water temperature was 12.5 °C and ranged from 10 to 16 °C, reaching the extremes only for short periods (1–2 h) on some days. Tanks were cleaned regularly to remove algae and debris. Water changes in the small tanks were completed every two weeks by syphoning out 1.5 L and replacing with 1.5 L of freshly made treatment solution.

Experimental Measurements

Germination (% per small tank) was recorded daily for the full seven weeks including Dormancy and Germination periods. Minimum time to germination (days) was calculated as the number of days from the start of the treatment phase for the first seed to germinate and average time to germination (days) was the mean for all seeds that had germinated in that small tank by the end of the experiment. Seedling establishment (%) was recorded at the end of the experiment as a percentage. The leaf extension of seedlings was recorded fortnightly by measuring the length from the end of the leaf to the cotyledon. The number of branches was recorded, and the length of each branch recorded (distance from the tip of the branch to the main leaf), and these lengths summed for each plant. The summed growth per seed was expressed as an extension rate based on the time from seed germination (mm day^{-1}), and averaged for all seedlings per small tank. At the end of the experiment, all seedlings were separated into above (leaves) and below (roots) ground material, dried at 60 °C for 48 h and weighed. A growth rate was calculated for the above, below and total biomass for each seedling (mg day^{-1}) and then averaged for each small tank.

Statistical Analyses

Response variables were analysed using permutational analysis of variances (PERMANOVA) to determine the main and interactive effects of Estuary, the Dormancy salinity and the Germination salinity. Factors were as follows: Estuary (fixed), Block nested within Estuary (random, 6 levels, 3 per Estuary), Dormancy salinity (fixed) and Germination salinity (fixed). No seeds germinated in the 80 salinity treatments so these data were excluded from the analyses. Where Block was statistically non-significant ($p > 0.05$), this factor was removed and the data 'pooled' following Underwood (1990). Homogeneity of multivariate dispersions was checked with PERMDISP on the raw data for all response variables. If PERMDISP not met ($p < 0.05$), then transformations were applied and PERMDISP was re-run. Above-ground biomass was the only variable to require transformation (square root). Thus, statistical significance was set to $p < 0.05$ for all analyses. Post hoc pairwise tests were used to identify the significantly different levels within each significant main effect or interaction. The analyses were conducted using PRIMER v7 software (Clarke and Gorley 2015) with the PERMANOVA add-on module (Anderson et al. 2008).

Results

Approximately 93% of all seeds found at the seagrass sampling sites were *R. polycarpa* with the remainder *R. megacarpa*, so only *R. polycarpa* is reported on. The average viability of *R. polycarpa* seeds 47.7 ± 6.3%. Across both estuaries and all months, the average seed density ranged from 9 ± 6 to 24 ± 14 m^{-3}, average germling density ranged from 0 to 0.1 ± 0.2 m^{-3} and average seedling density ranged from 0 to 6 ± 8 m^{-3} and generally, densities were higher at < 5 cm depth (Supplementary Fig. 1, Fig. 2).

Porewater Salinity Conditions over the Life Cycle of Seagrasses and in Seasonally Dry Areas Supporting Early Life Stages

Porewater salinity trends were similar across the two estuaries, declining over winter-spring (August to September/October) and then increasing over spring into summer (November to January; Fig. 2). The timing and magnitude of minima varied between the two estuaries, reaching 5 in September in the Vasse estuary and 10 in October in the Wonnerup estuary (Fig. 2). Maxima occurred in January in the Vasse and Wonnerup reaching 40 and 45, respectively (Fig. 2). Dormant seeds dominated across both estuaries, months and depths (Fig. 2). Seedlings were present across most depth intervals and months, except December (Fig. 2). Germlings generally occurred in < 10-cm depth, earlier months (July to October) and in the Wonnerup (Fig. 2).

At the sites which seasonally dried, porewater salinity varied between seasons, across the hydrologic gradient within the sites and with sediment depth (Fig. 3). In both seasons, maximum porewater salinities occurred above the water's edge but were higher at the end of the dry season (max. ~ 110) compared to the start (max. ~ 70) (Fig. 3). The depth of these maximum values differed between seasons, occurring deeper (5–10 cm) at the end of the dry season and shallower (0–5 cm) at the start of the dry season (Fig. 2). In submerged sediments, surface and porewater salinity (0–5 cm) varied more at the start of the dry season when the surface water had a higher salinity (42 at the surface versus 30 in the pore water) compared to the end of the dry season when the porewater salinity was higher (52 versus 60, respectively). In submerged sediments, there was greater variation in porewater salinity with increasing depth at the end of the dry season (60 to 85) compared to at the start of the dry season (25–35, Fig. 3).

Experimental Study

Seed Germination (%)

Across estuaries and treatments, germination rates ranged between 0 and 77% (Fig. 4A). Germination occurred in the 10 (41–77%) and 20 (9–70%) Germination treatments but not in the 80 treatments (Fig. 4A). There was a significant effect of each factor Estuary, Dormancy salinity and Germination salinity on germination rate, with no significant interactions (Fig. 4A; Table 2). The sum of squares (SS) indicated that Dormancy salinity (SS = 10,809) explained 51% of the total variation (SS total = 21,265). Significantly fewer seeds from the Vasse germinated compared to the Wonnerup (34% v 42%). Seeds previously exposed to 15 had significantly lower germination (23 ± 15%) compared to those previously exposed to 60 (49 ± 25%) or to 150 (43 ± 22%) (Fig. 4A). Germination was significantly higher for seeds transferred into 10 (66 ± 8%) compared to those transferred into 20 (49 ± 16%).

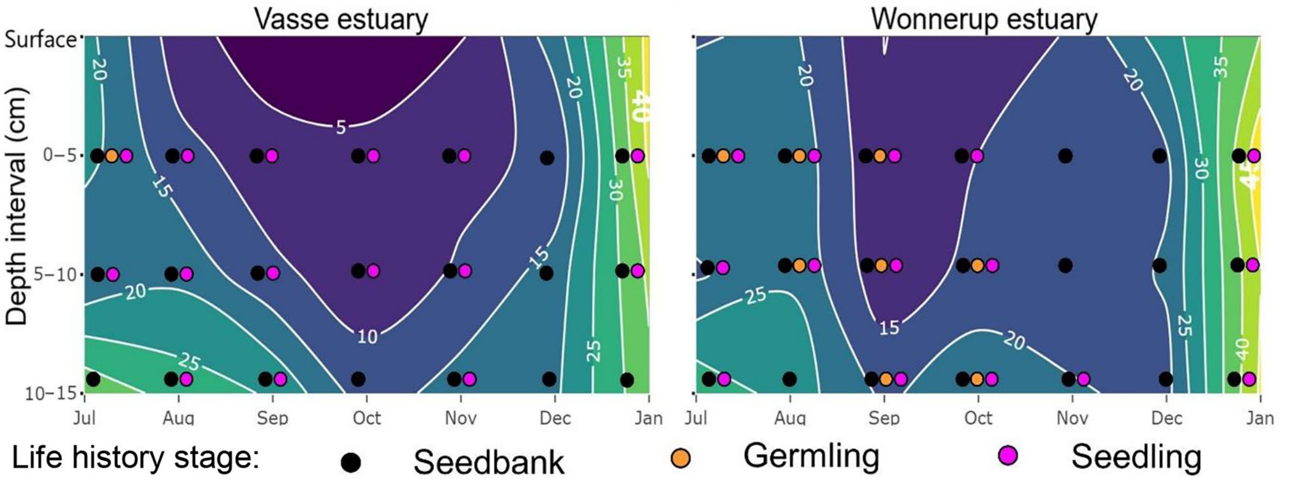

Fig. 3 Mean porewater salinity () in the surface water and in the sediment at three depth intervals: 0–5 cm, 5–10 cm and 10–15 cm in inundated areas. Each month, from July 2019 to January 2020, one replicate core was collected at each site in the Vasse estuary ($n=3$) and the Wonnerup estuary ($n=3$), southwestern Australia. Circles indicate presence of early life history stages of *Ruppia* spp

Mean Time and Minimum Time to Germination (Days)

The average time to germination was between 13 ± 2 and 29 ± 3 days (Fig. 5B). There was a significant effect of Estuary and Germination salinity on the mean time to germination (days) but no effect of Dormancy salinity and no significant interactive effects (Table 2), with Germination salinity explaining 41% of the variance. Seeds from the Vasse estuary germinated significantly faster (18 d) than those from the Wonnerup (22 d; Fig. 5B). Seeds germinated significantly quicker when transferred into 10 (17 days) compared to 20 (24 days).

The minimum time to germination ranged from 8 ± 0.3 days to 26 ± 4.5 days (Fig. 5C) and was significantly affected by a Dormancy × Germination salinity interaction ($p=0.0024$, Table 2). For seeds subjected to the 10 Germination treatment, those previously exposed to 15 (11 days) or 60 (10 days) Dormancy treatment germinated in significantly less time than those exposed to 150 (13 days). In contrast, for seeds subjected to the 20 Germination treatment, those previously exposed to 60 (14 days) germinated in significantly fewer days than those previously exposed to 15 (26.4 days) or 150 (18.5 days) (Fig. 5C).

Seedling Establishment (%)

On average, seedling establishment occurred for 23–77% of seeds that had germinated (Fig. 4D). Seedling establishment

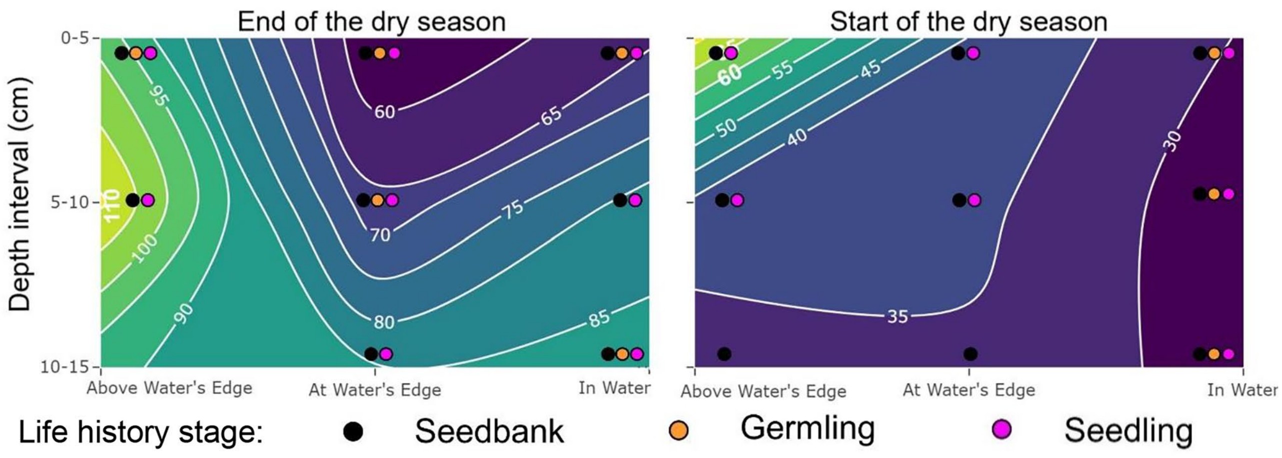

Fig. 4 Mean porewater salinity () in the sediment at three depth intervals: 0–5 cm, 5–10 cm and 10–15 cm along a gradient of drying. Three replicate cores were collected at each point along the gradient at two sites ($n=6$) at the end (June 2019) and start of the dry season (January 2020) within the Vasse estuary, southwestern Australia. Circles indicate presence of early life history stages of *Ruppia* spp

Table 2 Results from PERMANOVA statistical tests examining the influence of location (Location), Dormancy salinity (15, 60, 150) and Germination salinity (Treatment: 10, 20, 80) average seed germination (%) and average minimum number of days to germination. Significant effects ($p \leq 0.05$) are indicated in bold text

Source	*df*	*F*	*p*	*df*	*F*	*p*
Average seed germination (%)				**Mean time to germination (days)**		
Estuary	1	4.92	**0.04**	1	9.29	**0.007**
Dormancy	2	23.61	**0.0001**	2	2.91	0.07
Germination	1	10.07	**0.004**	1	34.55	**0.0001**
Est × Dor	2	0.65	0.53	2	1.27	0.30
Est × Ger	1	0.54	0.47	1	0.04	0.85
Dor × Ger	2	2.32	0.12	2	3.22	0.06
Est × Dor × Ger	2	0.10	0.91	2	1.18	0.32
Residual	24			23		
Total	35			34		
Minimum time to germination (days)				**Average seedling establishment (%)**		
Estuary	1	4.04	0.06	1	2.50	0.1
Dormancy	2	11.36	**0.0006**	2	17.84	**0.0001**
Germination	1	48.38	**0.0001**	1	16.19	**0.0003**
Est × Dor	2	0.43	0.7	2	1.01	0.4
Est × Ger	1	0.01	0.9	1	0.02	0.9
Dor × Ger	2	8.45	**0.002**	2	2.20	0.1
Est × Dor × Ger	2	0.18	0.8	2	0.02	0.9
Residual	23			23		
Total	34			34		
Average shoot extension rate (mm day^{-1})				**Average growth (mg day^{-1})**		
Estuary	1	1.11	0.3	1	0.0002	0.6
Dormancy	2	2.67	0.09	2	0.0004	0.6
Germination	1	14.36	**0.001**	1	0.0002	0.7
Est × Dor	2	3.90	**0.04**	2	0.0007	0.5
Est × Ger	1	0.26	0.6	1	0.0009	0.3
Dor × Ger	2	0.36	0.7	2	0.002	0.1
Est × Dor × Ger	2	0.46	0.6	2	0.0008	0.4
Residual	23					
Total	34					
Average above-ground growth (mg day^{-1})				**Average below-ground growth (mg day^{-1})**		
Estuary	1	3.17	0.09	1	0.04	0.8
Dormancy	2	0.61	0.6	2	0.49	0.6
Germination	1	0.77	0.4	1	0.0004	0.9
Est × Dor	2	0.70	0.5	2	0.48	0.6
Est × Ger	1	0.09	0.7	1	1.30	0.3
Dor × Ger	2	0.09	0.9	2	2.78	0.08
Est × Dor × Ger	2	0.41	0.7	2	0.55	0.6
Residual	22			22		
Total	33			33		

was not affected by the Estuary the seeds originated from with similar values recorded for the Vasse estuary (32 ± 6%) and Wonnerup (37 ± 6%) (Estuary, $p = 0.1255$, Table 2, Fig. 4D). There were significant main effects of Dormancy salinity ($p = 0.0001$) and Germination salinity ($p = 0.0003$) on seedling establishment. For Dormancy salinity, establishment was significantly higher for seeds previously exposed to 60 (46 ± 8%) than those previously exposed to 150 (37 ± 7%) which was significantly higher again than the 15 (21 ± 6%). For Germination salinity, more seedlings established in the 10 treatments (62 ± 4%) compared to the 20 treatments (41 ± 6%; $p = 0.0011$). Approximately 44% of the variance in seedling establishment was explained by the Dormancy group (SS = 8536) compared to 19% by the

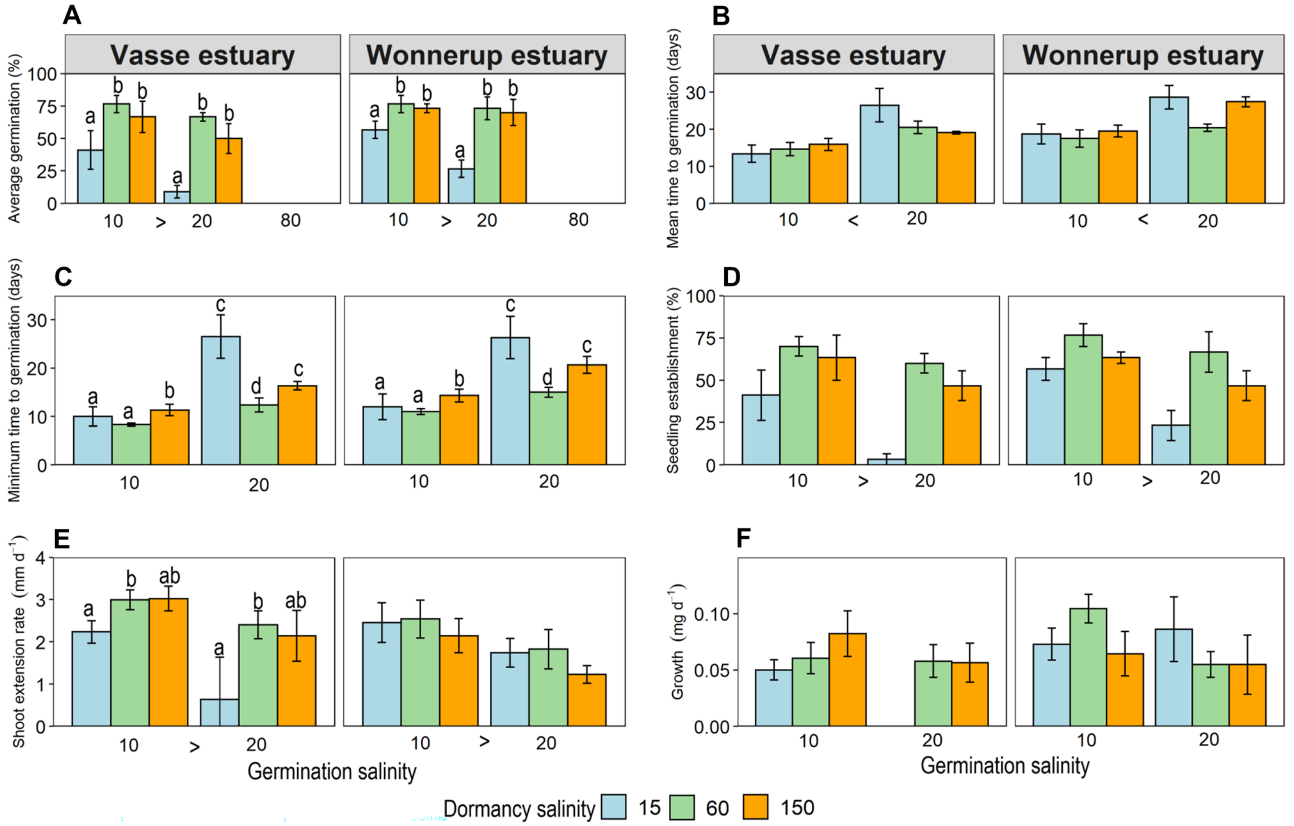

Fig. 5 Effect of Dormancy salinity (15, 60, 150) and Germination salinities (10 and 20) on germination processes (mean ± SE) of *Ruppia polycarpa* seeds collected from two estuaries in southwestern Australia. Seed germination (**A**), time to germination (**B**), minimum time to germination (**C**), seedling establishment (**D**), shoot extension rate (**E**), growth rate (**F**). Note that a Germination salinity of 80 was also used in the experiment but no seeds germinated so the group was excluded from the analyses. Letters represent the outcomes of pairwise PERMANOVA tests for significant differences between Dormancy groups. The symbols 'greater than' or 'lesser than' indicate the direction of the significant difference between seeds in the Germination salinity groups

Germination salinity group (SS = 3872) (total SS = 20,299) Fig. 5D.

Shoot Extension Rate (mm day−1) and Growth (mg day−1)

Shoot extension rates ranged between 1.2 ± 0.2 and 3 ± 0.2 mm day^{-1} (Fig. 4E). Germination salinity had a significant effect on shoot extension rate with higher rates for seedlings in 10 (2.61 mm day^{-1}) compared to those in 20 (1.77 mm day^{-1}) ($p = 0.0014$, Table 2). There was also a significant interactive effect of Estuary × Dormancy salinity on leaf extension ($p = 0.0369$, Table 2). Within the Vasse estuary, the shoot extension rate was significantly higher for seedlings previously exposed to 60 (2.70 mm day^{-1}) compared to those exposed to 15 (1.96 mm day^{-1}), but in the Wonnerup shoot extension rate was similar across all Dormancy salinity treatments. Like seedling establishment, Dormancy salinity explained more of the variance in shoot extension rate than Germination salinity (26% versus 14% respectively, Table 2). Seedling growth ranged from 0 to 0.14 mg day^{-1} (Fig. 4F) but the differences amongst Estuaries, Dormancy and/or Germination salinity groups were not significant (Table 2). The results for above-ground and below-ground growth were similar to total growth (Table 2).

Discussion

Understanding the environmental cues that initiate germination of foundation plant species is crucial for predicting how global change will affect their resilience (Cumming et al. 2017). Our findings show that porewater salinity dynamics are one such environmental cue, being critical for the release of seeds from dormancy and their subsequent germination success for the seagrass *R. polycarpa*. Two aspects of the porewater salinity dynamics appear to be important: the absolute salinities that seeds experience and a significant change in salinity. Provided salinity declined to low levels, either 20 or 10, seeds that were previously exposed to hypersalinities (150 or 60) germinated more quickly and more seeds germinated overall. Where the salinity only declined to 80 we saw no germination. These findings indicate a 'benefit' of exposure to hypersalinity during the dry season through enhanced seed germination if subsequent winter rainfall reduces salinity sufficiently. However, they also hint at potentially negative consequences for the seagrass if future climate change alters the timing or magnitude of salinity changes within estuaries.

Seasonal Drying and Wetting Creates Dynamic Porewater Salinity Regime Which Strongly Affects Germination

The conditions seeds are exposed to during dormancy can affect germination (Wetson et al. 2008). In this study, germlings were found across a range of salinity conditions in the sediment porewaters but predominantly during June to August, when they experienced a shift from hyper- to hyposalinity. Our experiment confirmed that this type of change in salinity enhanced germination, especially if seeds were transferred from hypersaline conditions to salinities less than 20. Such 'salt stimulation' has been reported for other *Ruppia* spp. (van Vierssen et al. 1984) and species occupying salt marshes (Woodell 1985). Exposure to high salinities and/or drying is considered beneficial for germination as it ruptures the seed coat and enables the uptake of water as soon as rainfall occurs and stimulates enzyme activity needed for embryonic growth (Leopold and Kriedemann 1975). Our observed lack of germination when seeds were transferred into the 80 treatment suggests that the 'benefit' of exposure to hypersaline conditions during dormancy will manifest only if salinity is then reduced sufficiently. Low salinities enhance the magnitude of germination (as shown in this study), lower abiotic stress (Touchette 2007) and enhance seedling survival (Strazisar et al. 2013a). Seagrasses evolved in freshwater environments and began colonising marine systems over 100 million years ago, so enhanced performance in low salinities may reflect their evolutionary history (Waycott et al. 2006). This may explain why in 10 compared to 20, we recorded greater overall germination, generally in less time and with higher seedling establishment and subsequent growth (as measured by leaf extension). Based on our findings, the optimum for *R. polycarpa* seeds and seedlings is likely closer to 10, which lies between 5 reported for *R. sinensis* (Gu et al. 2018) and 15 for *R. maritima* (Koch and Dawes 1991). Our findings also suggest that there is some sort of threshold of change in salinity for germination to occur and that this probably lies between 80 and 20, though we can be no more precise than that. Overall, these results confirm that porewater salinity dynamics are important drivers of seed dormancy release and germination of *R. polycarpa*.

Strategies Adopted at Early Life Stages Could Afford Resilience to Altered Salinity Regimes

Our observations of dormant seeds and germlings over the entire field sampling period and over a range of in situ salinities are indicative of a plant with strategies well suited to a highly variable environment. Only a proportion of *R. polycarpa* seeds germinated even though conditions were favourable which was evidenced by germlings also being present. The combination of a persistent seedbank and variation in the timing of dormancy release 'bet-hedge' against changing conditions changing before reproduction can successfully occur and reduces the risk of extinction (Gremer et al. 2016). Germination of *R. polycarpa* was not limited to a particular salinity, rather it occurred over a range of salinity conditions. Having general requirements for germination can increase the total number of individuals that reproduce and contribute to replenishing of the seedbank in habitats with unpredictable conditions (Barga et al. 2017) and under climate change (Gremer and Venable 2014). We also observed that *R. polycarpa* germlings first appeared in June, the start of the growing season and when salinity begins to decline, indicative of germination cuing (Donohue et al. 2010). Such early germination would increase the duration that seedlings were exposed to low salinity conditions, increasing the likelihood of completing their life cycle and reproducing (Strazisar et al. 2013b). By adopting a combination of bet-hedging and germination cuing, we conclude that *R. polycarpa* is adapted to tolerate variable salinity conditions. However, changes in conditions from climate change can disrupt bet-hedging strategies (Ooi et al. 2009). Therefore, we stress the need to investigate the mechanisms which uphold these strategies of *R. polycarpa* to improve our understanding of its ability to respond to future pressures.

In the experiment, no germlings occurred in 80 but, in the field, were found in a broad range of salinities from 60 to 110. Vollebergh and Congdon (1986) recorded germination of *R. polycarpa* in a Victorian saltmarsh in high salinities (40), again contrasting our experimental findings. These contrasting experimental and field observations might be explained by the nature of the salinity regimes in the different studies. In the experiment, seeds were exposed to pulsed salinity treatments, abruptly changing from hypersalinity to hyposaline conditions, whereas in the field salinity changed gradually over time. Gradual changes in salinity allow for osmotic adjustment and can extend the salinity tolerance of seedlings (Strazisar et al. 2013a) and adults (Griffin and Durako 2012). Another plausible explanation relates to the highly heterogenous nature of sediments at small spatial scales. It is possible that there were variations in salinity at small spatial scales that were not captured by the temporal and spatial resolution of monitoring adopted here (Stockdale et al. 2009), such as from fresh localised groundwater inflow. This small-scale variation in porewater salinity is supported from the field studies where, at some times of year, the porewater salinity was lower at depth. Climate change will induce both gradual and acute changes to salinity (Tweedley et al. 2019). Further studies comparing the effects of both scenarios will improve our understanding of the implications for *R. polycarpa*.

Modifications to Salinity Regimes Under Global Change May Shift Germination Timing

Our results confirmed that, under current conditions, *R. polycarpa* displays a winter annual reproductive strategy and germination behaviour that is influenced by porewater salinity, characteristics that make it susceptible to future changes in rainfall and salinity regimes. For Mediterranean-climate regions, predicted climate change impacts include reductions in winter rainfall and more intense summer rainfall events (Andrys et al. 2017), with both likely to alter the temporal dynamics of porewater salinity. Whilst we showed that a decline to 20 is adequate to trigger germination, salinity may not decline to this level as winters become drier, lowering seed germination and seedling survival (Kim et al. 2013). This scenario is more applicable to systems with relatively low freshwater input, such as the Wonnerup estuary in our study. Reductions in salinity may also occur later, delaying germination to later in the winter and requiring the life cycle to be completed more quickly to ensure reproduction occurs before the system dries. Alternatively, extreme summer rainfall may dramatically reduce salinity (Du et al. 2019) causing seeds to germinate far earlier than normal, with the risk of germlings dying if the subsequent conditions are unfavourable. The implications of such shifts in germination timing are likely to depend on how the effects manifest in subsequent life cycle stages. For alpine species, a shift in the timing of germination posed the greatest risk to seedlings (Mondoni et al. 2012). The consequence of changes in the timing of germination was not addressed in this study and require further research.

Site-Level Differences Show Variation in Germination Success Could Confer Resilience

Site-level differences in germination responses to environmental conditions are useful to know to improve predictions of species' responses to climate change (Koch and Seeliger 1988). In our study, site-specific differences manifested in two ways. Seed germination was higher in the Wonnerup estuary but seeds from the Vasse estuary germinated in less time on average. Greater seed germination would increase the likelihood of seedlings becoming adults and producing propagules provided conditions were favourable (Olesen and Sand-Jensen 1994). However, climate change can shorten 'windows of opportunity' for critical life history transitions (Mondoni et al. 2012). Thus, the strategy of earlier germination may be more advantageous in the context of climate change since it would enable plants to reach sexual maturity quicker and reproduce for longer periods of time, promoting persistence in subsequent seasons (Donohue et al. 2005). Seeds from the Vasse estuary appear to have traits that could confer greater resilience to future environmental change. The mechanism explaining these differences is unclear since the porewater salinity regimes of both estuaries were similar. Future work should seek to increase understanding of seed ecology as well as identifying the mechanisms influencing these differences to test future-proofing methods for macrophyte restoration (Wood et al. 2019).

Sediment Seedbank Assessment Reveals Dynamic Salinity Regime and Potential Bottlenecks to Seagrass Resilience

Quantifying sediment microenvironmental conditions that can affect early life stages is important for understanding the resilience of seagrass and salt marsh in variable salinity environments (Wiegert et al. 1983). In estuarine systems that support annual populations, our results revealed that critical life history stages of seagrasses (seeds, germlings and seedlings) are exposed to very different salinity conditions in the sediment compared to the overlying surface water. To our knowledge, the present study is one of the few investigations to describe the porewater salinity conditions that early life history stages of seagrass are exposed to and relate this to germination. Porewater analyses revealed a highly dynamic and unique salinity regime that did not always reflect surface salinity conditions. For instance, at particular times and locations there was variation in salinity through the sediment profile and the nature of these patterns varied over time. There did appear to be an overlap in the seasonal trends in surface and porewater salinity; both declined during winter following rainfall however, porewater salinity can also be affected by other factors such as subsurface flow (Moffett et al. 2012). There is a need for future ecological modelling of estuarine responses to climate change to factor in porewater concentrations and conditions rather than simply assuming surface water conditions are all that plants experience and are responding to.

Conclusions

This study is one of the few investigations to describe porewater salinity conditions and provides evidence that early life history processes of seagrass, including seed dormancy and germination, are synchronised to the seasonal drying and wetting of highly seasonal estuarine habitats. A shift from higher to lower salinity was an important environmental cue that enhanced the germination of *R. polycarpa* seeds but only if salinity was reduced enough. On this basis, we predict that declining winter rainfall could reduce germination however, extreme rainfall events in summer may induce summer germination with potentially negative consequences. Germination was not restricted to a particular salinity but occurred over a range of salinities, indicative of bet-hedging. This strategy could enable regeneration even under increasing salinities associated with warming. Assessments of the performance of later life history stages following delayed or unseasonal germination are needed to fully understand whether populations can reproduce and persist as salinity becomes more erratic.

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Author Contribution CW, KM and PL made substantial contributions to conception and design, analysis and interpretation of data. CW led the field and laboratory data collection and drafting and finalising the manuscript. SS made substantial contributions to data acquisition in the field and laboratory, conception and design and reviewing the manuscript. JB made substantial contributions to data acquisition in the laboratory and reviewing the manuscript.

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Declarations

Conflict of Interest The authors declare no competing interests.

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