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Seed dormancy and germination of *Halophila ovalis* mediated by simulated seasonal temperature changes

John Statton [a, *], Robert Sellers [a], Kingsley W. Dixon [a, b, d], Kieryn Kilminster [a, c], David J. Merritt [a, d], Gary A. Kendrick [a]

[a] School of Biological Sciences and UWA Oceans Institute, Faculty of Science, University of Western Australia, Crawley, 6009 Western Australia, Australia
[b] Department of Environment and Agriculture, Curtin University, Bentley, Perth, Western Australia, Australia
[c] Department of Water and Environmental Regulation, Government of Western Australia, Locked Bay 33, Cloisters Square, Perth, Western Australia 6850, Australia
[d] Kings Park and Botanic Garden, Kings Park, 6005 Western Australia, Australia

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ABSTRACT

The seagrass, *Halophila ovalis* plays an important ecological and sediment stability role in estuarine systems in Australia with the species in decline in many sites. *Halophila ovalis* is a facultative annual, relying mainly on recruitment from the sediment seed bank for the annual regeneration of meadows. Despite this, there is little understanding of seed dormancy releasing mechanisms and germination cues. Using *H. ovalis* seed from the warm temperate Swan River Estuary in Western Australia, the germination ecology of *H. ovalis* was investigated by simulating the natural seasonal variation in water temperatures. The proportion of germinating seeds was found to be significantly different among temperature treatments ($p < 0.001$). The treatment with the longest period of cold exposure at 15 °C followed by an increase in temperature to 20–25 °C (i.e. cold stratification) had the highest final mean germination of 32% and the fastest germination rate. Seeds exposed to constant mean winter temperatures of 15 °C had the slowest germination rate with less than two seeds germinating over 118 days. Thus temperature is a key germination cue for *H. ovalis* seeds and these data infer that cold stratification is an important dormancy releasing mechanism. This finding has implications for recruitment in facultative annual species like *H. ovalis* under global warming since the trend for increasing water temperatures in the region may limit seed-based recruitment in the future.

1. Introduction

Sexual reproduction plays a vital role in population persistence, expansion and regeneration of many seagrass populations (Hovey et al., 2015; Kendrick et al., 2012, 2016; Strazisar et al., 2015). Therefore, incorporating the dynamics of sexual reproduction and early life-history processes in conservation and restoration strategies underpins effective management (Hovey et al., 2015; Kilminster et al., 2015). For many seagrass species, the small size of seeds and seedlings, their rapid growth (e.g. *Halophila* sp.) and spatial and temporal variability *in situ* often affects our ability to directly observe early life stages. As a result, for most seagrass species there is little understanding of the environmental conditions that affect the timing, success rates and relative importance of recruitment from seeds.

Halophila ovalis is widely distributed in coastal waters and estuaries throughout the Indo-Pacific and western Pacific Ocean, as well as coastlines bordering the Indian Ocean (Short et al., 2007). It is a facultative annual (predominantly replaces senesced genets via seed) with seasonal colonisation and, though a vigorous coloniser, is highly sensitive to disturbance (Kilminster et al., 2015). Disturbance-driven species like *Halophila ovalis* are typically highly fecund with conservative estimates of 900 seeds m^{-2} potentially entering the sediment seed bank each year in southwest Australia (Kilminster et al., 2014; Kuo and Kirkman, 1992). In tropical regions, seed bank recruitment has been observed to play an important role in the re-colonisation of *H. ovalis* meadows when vegetative growth is experimentally restricted (Rasheed, 2004) or when there is natural widespread loss of adult populations from catastrophic events such as cyclones and flooding (Preen et al., 1995). In some

* Corresponding author..
E-mail address: john.statton@uwa.edu.au (J. Statton).

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cases *H. ovalis* seeds have remained viable and germinated 18 months after a flood event (Campbell and McKenzie, 2004). In addition, *H. ovalis* ramets are short-lived with fast growth rates enabling colonisation of several square metres within a growing season from a single seeding event indicating a critical role of early life-stages supporting localised occurrence of *H. ovalis* populations. However, when seeds enter the sediment the environmental conditions that influence early life-history processes (e.g. seed dormancy and germination) are poorly understood.

The germination requirements of *Halophila* seeds have been investigated in only a few studies and exposure to light is the most commonly reported factor for inducing germination (reviewed in Orth et al. (2000)). For example, *Halophila decipiens* seeds have been shown to persist in culture in the dark for 11 months (McMillan and Soong, 1989), and *H. engelmannii* for 24 months (McMillan, 1991), germinating only when exposed to light. In *Halophila ovalis*, light wavelengths also influence germination, with red light resulting in a 65% increase in seed germination compared to blue light (Strydom et al., 2017). However in each study, germination after exposure to light has been asynchronous and variable; from 2 to 23 days in *H. decipiens* (McMillan, 1988b; McMillan and Soong, 1989), and from 3 weeks to 6 months in *H. ovalis* (Kuo and Kirkman, 1992). Whether such marked variation in germination traits is due to differing dormancy states, or instead a lack of appropriate environmental conditions for germination is unclear. Also it is possible that the observed differences in timing of germination within and amongst seed populations may be attributed to intra-specific genetic differences or to different phenotypes or eco-types (e.g. marine vs estuary populations) that have been shown to influence other physiological traits of *H. ovalis* (Benjamin et al., 1999).

In terrestrial and aquatic plants, seed dormancy and germination can be influenced by many environmental factors including moisture, temperature, light, salinity and nutrient levels (Baskin and Baskin, 2014). Seed dormancy and germination can be viewed as two distinct (but linked) physiological processes, with regulation of these physiological processes being influenced by the external environmental conditions (Thompson and Ooi, 2010; Vleeshouwers et al., 1995). In terrestrial plants, the primary abiotic environmental factors that regulate dormancy are moisture and temperature, and as seeds lose dormancy, the environmental conditions under which seeds can subsequently germinate can become broader (Baskin and Baskin, 2014; Vleeshouwers et al., 1995). Temperature in particular has a considerable influence on the dormancy status of terrestrial and marine plant seeds and their ability to germinate (Kendall and Penfield, 2012; Moore et al., 1993; Probert and Brenchley, 1999). The loss of dormancy in moist seeds at temperatures of c. 0–10 °C (cold stratification) or at temperatures of ≥15 °C (warm stratification) is recognized as releasing physiological dormancy in seeds of many terrestrial species (Baskin and Baskin, 2014). But it is unknown how cold or warm stratification, processes that simulate seasonal changes in water temperature, will influence seed germination of *Halophila ovalis.*

Halophila ovalis is a foundation species of seagrass within the Swan River Estuary, Perth, Western Australia, but its coverage may have declined by more than one-third over the last 30 years due to anthropogenic impacts such as physical disturbance, changes in water and sediment quality, and climate (Kilminster et al., 2014) as the estuary lies within a highly urbanised environment. While there is a reasonable understanding of ecological processes in the Swan River Estuary that influence the adult standing stock of *H. ovalis* (Hillman et al., 1995; Kilminster et al., 2014), effects of these environmental processes on early life-history stages are less well known. This bias in understanding has recently been found to under-represent the dynamic nature of this genus (Hovey et al., 2015; Kilminster et al., 2015) and potentially limit the effectiveness of present management strategies. As with other opportunistic species, sexual reproduction is likely to be a vital step in the persistence of *H. ovalis* populations and early developing seedlings are potentially more sensitive to adverse conditions than adult plants and dormant seeds (e.g. *Ruppia maritima,* Strazisar et al. (2015)). Therefore, a better understanding of the timing of seedling presence could improve management practices for this species and facilitate the use of seed in restoring areas depleted of *Halophila*.

The aim of this study was to investigate the germination requirements of *Halophila ovalis* seeds collected from the Swan River Estuary. Our germination study focussed on the effect of temperature changes that emulate the seasonal differences in water temperature within the Swan River Estuary. We hypothesised that after seed release from the fruit (prior to winter), seeds would remain dormant and not germinate during unfavourable growing conditions for seedlings (ie. during winter), and that germination would be cued to coincide with increasing water temperatures in spring/summer.

2. Methods

2.1. Collection of seeds

Halophila ovalis fruit production in the Swan River Estuary generally occurs between February and May (Kilminster et al., 2014). Mature fruits were collected from the western side of Point Roe in the Swan River Estuary, Western Australia (Rocky Bay – Supplementary Information Fig. 1) on the 20th May 2014. Fruit maturation was followed for several weeks and fruits were assessed as mature based on a yellow colouration (versus green and small when immature), visibility of seeds within the fruit (fruit becomes more transparent with maturity) and the ease with which they detach from rhizomes when rhizomes were gently agitated by hand. For collection, intact fruits were dislodged from rhizomes by agitating (gently massaging) rhizomes by hand. Once dislodged, fruit were positively buoyant and floated to the surface. Floating fruit were collected in a net or sieve. Collected fruits were then placed in an insulated container filled with river water and transported back to the lab (20 min) for immediate seed extraction. Seed extraction followed a similar methodology to Statton et al. (2013) but with some modifications due to the small size of the seeds (Fig. 1). *Halophila ovalis* seeds are approximately 1 mm at maturity and both the seeds and the fruits (after dehiscence) are negatively

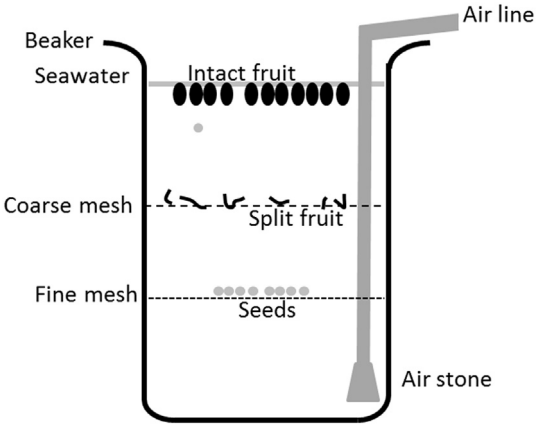

Fig. 1. Seed extraction set-up: a – air-line to air pump; b – coarse mesh to capture dehisced fruit; c – fine mesh to capture released seeds.

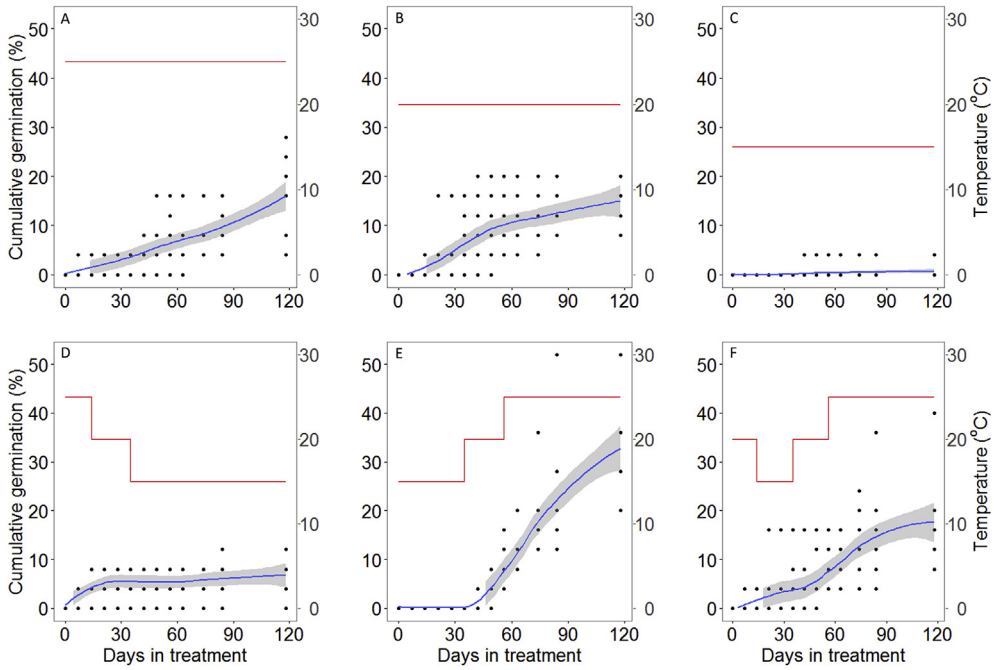

Fig. 2. Temperature treatments (red) with their associated germination responses (blue). Treatments A [25 °C], B [20 °C] and C [15 °C] are static temperature controls. Treatments D, E and F reflect aspects of annual temperature cycle: D - cooling temperatures into winter [25-20-15 °C]: E - winter cold stratification entering into spring and summer [15-20-25 °C]: F – temperature changes between seasons [20-15-20-25 °C]. Loess locally weighted linear regression was used to plot cumulative germination percentage and define 95% confidence intervals in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

buoyant. To separate seeds from dehisced fruits without excessive manual handling, fruits were placed in modified 2L beakers. Each 2L beaker was fitted with a coarse and a fine mesh (inert material) barrier. The coarse mesh (1.2 mm) was positioned at the 1.5 L graduation and fit neatly so that there were no gaps on the beaker walls. The fine mesh was fitted in a similar manner but at 0.5 mL graduation (ie. below the coarse mesh). An aeration line (5 mm diameter) was threaded through each mesh (ie. 5 mm hole perforated in each mesh for the air-line) and an air stone was placed on the bottom of the beaker below the fine mesh. Collected fruits (~250 with 8–12 seeds per fruit) were portioned between several of these modified 2L beakers filled with river water from the Swan River Estuary. Fruits were added to the surface of the beaker where they remained buoyant until the agitation by aeration caused fruit to dehisce (Statton et al., 2013). Dehisced fruit were collected in the coarse mesh and seeds were collected in the fine mesh (ie. they passed through the coarse mesh). Every 2–3 h, seeds and fruit were removed and only the mesh was handled to collect the seeds preventing the need for manual seed handling. Dehisced fruit were discarded. The seed extraction process continued for 36 h after fruit collection and greater than 90% of seeds dehisced within that time (~2000 seeds). All beakers were maintained in incubators at 20° C simulate mean Swan River Estuary water temperature in shallow waters at the time of collection with a light:dark photoperiod of 12:12 h at 40 μ mols photons m^{-2} s^{-1} the compensating irradiance for *H. ovalis* adult plants (Hillman et al., 1995). We used compensating irradiance rather than saturating irradiance to limit the rate of algal growth whilst still providing sufficient light to elicit a light response.

2.2. Experimental design and set-up

To determine the effect of simulated seasonal temperature changes in the Swan River Estuary on *H. ovalis* seed dormancy status and germination, we developed temperature treatments based on seasonal differences in mean water temperature sampled within the Swan River Estuary. Average weekly water temperature data was collected from the Swan River Estuary in 2013 (DoW, 2013). Raw data was then rounded to the nearest 5 °C to define treatment temperatures that reflect seasonal variation in water temperature (Supplementary Information Fig. 2). Seeds incubated at constant temperatures of 25, 20, or 15 °C (treatments A, B and C, respectively) for the entire 118 days served as controls. A 'move-along' experimental approach (Baskin and Baskin, 2004) was designed comprising three treatments (D, E and F) that simulated seasonal temperature cycles, compressed into approximately four months (118 days) (Fig. 4): D – a sequence of 25–- 20 - 15 °C (simulating summer through to winter conditions); E – a sequence of 15–20–25 °C (simulating winter through to summer conditions); F – a sequence of 20–- 15–20–25 °C (simulating autumn through to the following summer conditions). Twenty-five seeds were carefully pipetted into 120 ml specimen jars filled with Swan

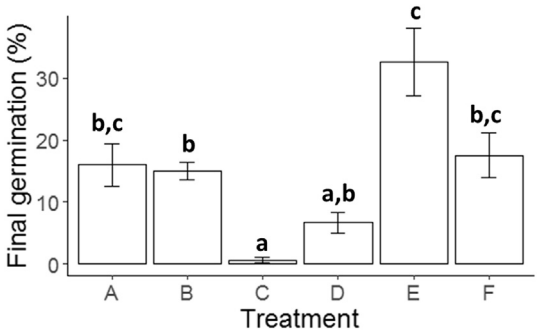

Fig. 3. Final germination percentages for seeds incubated within 'Static' temperature treatments A [25 °C], B [20 °C] and C [15 °C]: and 'move-along' temperature treatments; D [25-20-15 °C], E [15-20-25 °C] and F [20-15-20-25 °C]. Letters above each column denote significant differences ($p < 0.05$).

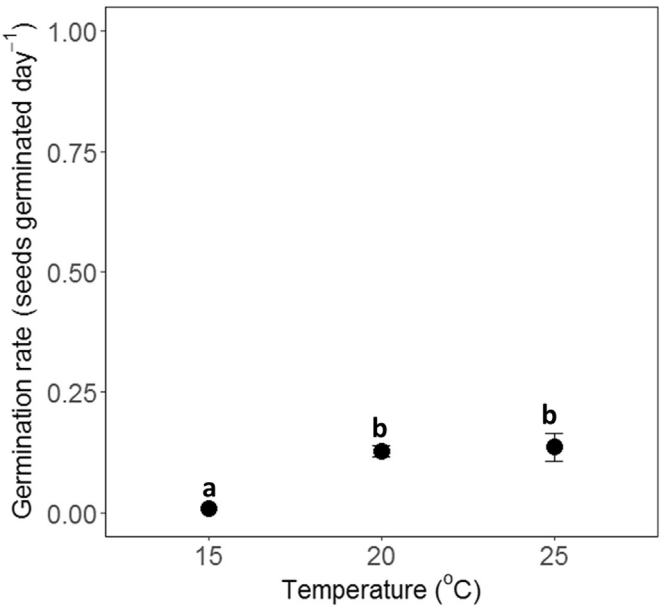

Fig. 4. Mean germination rates and 95% confidence intervals of seeds incubated within 'Static' temperature treatments A [25 °C], B [20 °C] and C [15 °C] Letters above each point denote significant differences ($p < 0.05$).

River Estuary water. Eight replicate jars (48 jars with 1200 seeds in total) were then randomly assigned to each temperature treatment. Water within jars was changed weekly with Swan River Estuary water to avoid possible build-up of anaerobic conditions and growth of algae. Replacement water was incubated to the temperature of each treatment prior to exchange. Germination (counts) was defined as emergence of either the radicle or shoot and was counted weekly for the first 12 weeks and every 10–14 days for the remaining 5 weeks.

2.3. Statistical analysis

Transformation of germination count data was avoided following the recommendations of Erceg-Hurn and Mirosevich (2008) and O'Hara and Kotze (2010). In all cases residuals were tested for normality using the Shapiro-Wilks test in the 'stats' package (R Core Team, 2014) in R, and heteroscedasticity using the Breush-Pagan test in the 'lmtest' package (Hothorn et al., 2017). We used Loess (locally estimated scatterplot smoothing) in R 'stats' package (R Core Team, 2014) to visually assess the relationship between the two variables; cumulative germination and number of days, for each temperature treatment (A, B, C, D, E, F). Confidence intervals were calculated using bootstrapping with 100 000 re-sampling iterations. Because data were non-normally distributed we used non-parametric tests to analyse the data (Cribbie et al., 2007). Brunner-Dette-Munk test rank based permutations test was used to compare final germination percentages using the BDM.test function of the 'asbio' package (Aho, 2015) in R v3.1.2 (R Core Team, 2014). Since the treatment factor had more than two levels (i.e., Treatments [A, B, C, D, E, F]), multiple comparisons were performed with the nparcomp function of the 'nparcomp' package (Konietschke et al., 2015) in R. To compare germination rates, expressed as the number of seeds germinating per day within a temperature interval, we performed a Kruskal Wallis rank sum test using the kruskal.test function of the 'PCMCR' package (Pohlert, 2014). We tested differences in germination rates amongst 'static' temperature treatments, A, B and C; and germination rates at each temperature interval within each 'move-along' treatment (D, E, and F). When a significant difference was detected we applied a HSD Tukey posterior pairwise analysis (nparcomp, R package) to test for differences between treatments.

3. Results

3.1. Cumulative germination

In all temperature treatments seed germination was non-synchronous though seeds showed clear germination responses to changes in temperature (Fig. 2). The treatment with the longest period of cold stratification followed by an increase in temperature (Treatment E) had the highest cumulative mean germination (Fig. 2). The 'Static' 15 °C treatment (Treatment C) had the lowest cumulative mean germination (Fig. 2). Within 'move-along' treatments (D, E but not F), seed germination responded to changing temperature regimes (Fig. 2), showing a decrease in germination when exposed to 15 °C and an increase in germination when exposed to 20 and 25 °C.

3.2. Final germination percentage

Final germination percentages were statistically significantly different between temperature treatments (Table 1, $p < 0.001$, Fig. 3). Seeds incubated at 15 °C for the 118 days experimental duration showed less than 1% germination. Seeds incubated at 20 °C and 25 °C germinated slowly, reaching 15 and 17% germination, respectively, after 118 days. In the 'move-along' temperature treatments, final germination was greatest in those treatments where seeds were exposed to a period of cool temperatures, followed by warm temperatures (Treatments E and F). Seeds incubated under the Treatment E regime (i.e. those that received the longest period of cold stratification) had the highest mean final germination for all treatments at 32%, with a maximum in one replicate of 52% (Fig. 3).

3.3. Germination rates

Germination rates at 'Static' incubation temperatures were significantly different between the highest (20 °C and 25 °C) and lowest (15 °C) 'Static' temperatures (Table 2, $p < 0.001$, Fig. 4). Germination rates at 'Static' 15 °C were low, with two seeds germinating over the 118 day experimental period (Fig. 4). Seeds

Table 1
One-way Brunner-Dette-Munk test for differences in final germination percentages between temperature treatments (A, B, C, D, E, F).

	df 1	df 2	F*	P(F > F*)
Treatment	3.69	28.24	13.17	5.34×10^{-6}

df 1 = degrees of freedom of the factors (Treatment).
df 2 = degrees of freedom of the whole model.
F* = non-parametric ANOVA-type F statistic.
(F > F*): p value; $\alpha = 0.05$.

Table 2
Kruskal Wallis rank sum test for germination rates, expressed as the percentage of seeds germinating per day within a temperature interval. We tested differences in germination rates amongst 'Static' temperature treatments, A (25 °C), B (20 °C) and C (15 °C); and germination rates at each temperature interval within each 'move-along' treatment (D [25-20-15 °C], E [15-20-25 °C], and F [20-15-20-25 °C]).

	Chi ^{2}	df	p
Static	14.86	2	<0.001
Treatment D (25-20-15 °C)	6.18	2	0.05
Treatment E (15-20-25 °C)	14.45	2	<0.001
Treatment F (20-15-20-25 °C)	6.22	3	0.10

incubated at 'Static' temperatures of 20 and 25 °C showed higher germination rates, with about one seed germinating every five-six days, respectively (Fig. 4).

Within 'move along' treatments (Treatments D, E and F), germination rates at the end of each temperature interval were calculated. These analyses demonstrated that germination rate was greatest at 20 and 25 °C, but the rate of germination decreased suddenly at 15 °C (Fig. 5). In 'move-along' Treatment D, germination of seeds when moved from 25 °C through to 15 °C was negligible but statistically significant (Table 2, $p = 0.05$, Fig. 5).

In 'move-along' Treatment E, germination commenced once seeds were moved from 15 °C to 20 °C, and maintained a statistically significant high rate of germination when seeds were moved to 25 °C (Table 2, $p < 0.001$, Fig. 5). Seeds in 'move-along' Treatment F commenced germination at the first 20 °C interval but showed no change in germination rate when transferred to 15 °C. Following a transfer to the second 20 °C interval and then 25 °C, germination rate did not vary significantly (Table 2, $p > 0.05$, Fig. 5).

4. Discussion

Seasonal changes in water temperature experienced in the Swan River Estuary are important drivers of seed dormancy release and germination for *Halophila ovalis*. We found that *H. ovalis* seeds require a decrease in water temperature (to 15 °C), analogous to cold stratification during winter, to alleviate dormancy, followed by an increase in temperature to 20 °C (spring) – 25 °C (summer) to stimulate germination. As hypothesised, warmer temperatures (even without a prior period of cold stratification) induced higher germination rates in *H. ovalis* seeds – there was mean germination of 16% in seeds exposed to temperatures equivalent to mean spring and summer temperatures (20–25 °C, respectively), compared to less than 1% germination in seeds exposed to a mean winter temperature of 15 °C. These outcomes indicate temperature is a key germination cue for *H. ovalis* seeds from the Swan River Estuary and suggest cold stratification as an important dormancy releasing mechanism. This finding has implications for recruitment in facultative annual species like *H. ovalis* under global warming. However, we are still uncertain whether it is the absolute or relative change in temperature that is influencing seed germination.

Cold stratification is an important dormancy releasing mechanism in *H. ovalis* in the Swan River Estuary. While no previous studies have directly investigated the effect of stratification on the germination of *Halophila* seeds, McMillan (1988a) showed that seeds extracted in mid-winter had a mean germination of 94%, compared to 32% germination in mid-autumn, a result consistent with cold stratification within the sediment contributing to the increased germination. The positive effect of cold stratification on germination of *H. ovalis* seeds in this study suggests that previous germination studies on seagrasses in temperate regions using static temperature treatments may have under- or over-estimated germination rates occurring in the field. It has been assumed that burial of seeds inhibits germination through the insulation of the seed to environmental triggers (Mcfarland and Shafer, 2011). However, where studies have used seeds gathered from the sediment seedbank at different times for germination trials, the prior-exposure of seeds to different temperatures conditions may have influenced the dormancy status of the seeds, confounding the results (McMillan, 1988a). Also, seeds in marine and estuarine sediments are often exposed to a range of other conditions including anoxia and high ethylene concentrations. Anaerobic conditions have been shown to increase germination in *Zostera marina* and *Zostera capricorni* (Brenchley and Probert, 1998; Moore et al., 1993), and exposure to ethylene and other gases under anaerobic conditions also stimulates germination in some aquatic species, acting as a water depth-sensing mechanism (Baskin et al., 2003; Cross et al., 2014; KeÇpczyński and KeÇpczyńska, 1997). Such treatments are yet to be investigated for *Halophila*, and interactions between temperature-regulated dormancy loss and other environmental signals such as ethylene and sulphide warrant further investigation.

Seasonal fluctuations in water temperature appear to be important for cueing germination for some other seagrass species. For example, in seeds of the seagrass *Zostera marina*, cold storage at 4 °C for 8 weeks resulted in a seven-fold increase in emergence of seeds that were germinated at 13 °C (Tanner and Parham, 2010). In seeds of *Phyllospadix iwatensis*, germination commenced upon the cooling of water temperature to c. ≤10 °C, after 5 months of exposure to warmer water temperatures of c. 15–19 °C (Kuo et al., 1990). In another study on *H. engelmannii* growing in shallows in coastal regions of Texas, seeds that were collected in October germinated in light at 24–27 °C, 27–33 days after collection, but for seeds collected in November–May, germination began in light in the laboratory after 7–10 days. Seeds that were collected in June and kept in the dark at 24–27 °C for 18 weeks (late September)

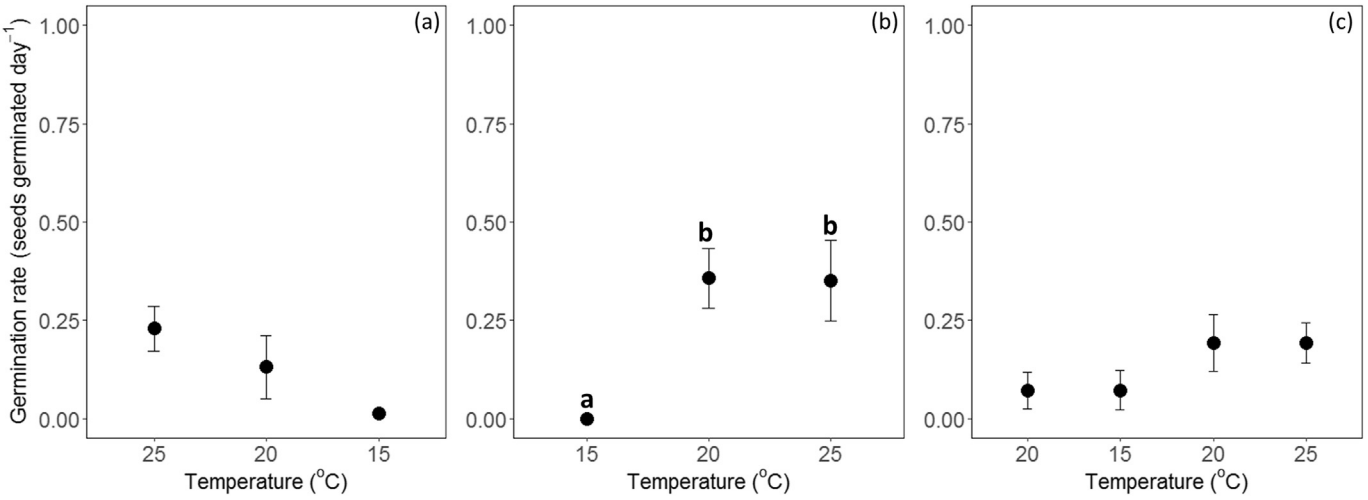

Fig. 5. Mean germination rates and 95% confidence intervals of seeds incubated at each temperature interval within each 'move-along' treatment. (a) D – a sequence of 25 –- 20 - 15 °C; (b) E – a sequence of 15–20–25 °C; (c) F – a sequence of 20- 15-20-25 °C. Letters above each point denote significant differences ($p < 0.05$).

germinated after 27–38 days in light, but seeds kept in the dark for 30 weeks began to germinate after 9–12 days (McMillan, 1988a). While these studies and our findings indicate the importance of seasonal temperature fluctuations in germination, for many seagrass species seasonality in germination response is not yet well defined.

In this study we limited pre-treatment effects by using freshly collected seeds that were carefully handled and tested fresh to remove artefacts that may confound germination outcomes. Previous practices or approaches for testing seed dormancy and germination of some seagrasses have led to uncertainty in the transferability of outcomes to natural conditions (Orth et al., 2000). Many germination trials have exposed seeds to one or several pre-treatments which can also influence dormancy and germination (Brenchley and Probert, 1998; Jewett-Smith and McMillan, 1990). For example, methods of *Halophila* seed collection have been variable and could influence the outcome of germination and dormancy tests. From thirteen published studies on *Halophila* sp., six gathered seeds from harvested fruit and the remainder either did not state the seed source or gathered seed of unknown age from sediments (Orth et al., 2000). Seeds gathered from sediments have been deposited for an unknown period and may have been exposed to a range of temperatures or other conditions that could influence germination rate (McMillan, 1988a). Use of seeds from the sediment seed bank are therefore compromised in terms of resolving ecologically significant thermal optima as seed will have experienced varying degrees of 'thermal pre-treatment' depending on the annual cohort of seed entering the sediment seed bank. Only one study (Bujang et al., 2008) germinated freshly collected *Halophila* seeds prior to entering the sediment seed bank though it was not quantitative and therefore inconclusive.

During this study we developed a number of methodological improvements and advancements which allow fruit collection of *Halophila ovalis* without harming adult plants as well as increasing the speed of collecting large quantities of intact fruit and subsequent rapid processing of fruit to extract seeds. Such advances could support greater restoration and management efforts utilizing *H. ovalis* seeds. Collection of sufficient quantities of seeds is often a major bottleneck to re-seeding programs for most seagrass species. In particular, caution has always prevailed when the collection method is destructive to the donor meadow. However, collecting in a less destructive manner, efficiently, may be an effective tool to develop a restoration or management program.

5. Conclusion

Increased germination of freshly collected *H. ovalis* seeds in response to a short duration (c. 4 weeks) of cold stratification suggests the seeds possess non-deep physiological dormancy (Baskin and Baskin, 2014). Results from this study suggest that changes in early life-history processes (seed dormancy and germination) closely follow the seasonal temperature changes in the Swan River Estuary. This information will be useful for improving our predictive capability to identify when different early life-history stages are most likely to be present within the estuary and for enhancing germination if seed is to be used in restoration programs (Strazisar et al., 2015). In addition, this finding has implications for the on-going regeneration of the species since the trend for increasing water temperatures as a result of a warming climate in the region may limit seed-based recruitment in the future. Future studies may need to investigate if more warm water tolerant forms of *H. ovalis* occur at lower latitudes along the western coastline of Australia that may provide suitable seed sources in the event of a collapse in recruitment in the Swan River estuary.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2017.08.045.

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