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Port Curtis Seagrass Seed Bank Density and Viability Studies - Year 3

Final Report

Reason CL, Rasheed MA, Carter AB & Jarvis JC

Report No. 17/40

Port Curtis Seagrass Seed Bank Density and Viability Studies - Final Report

A report for the Ecosystem Research and Monitoring Program

Report No. 17/40

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SUMMARY

Seagrass seeds and seed banks (seeds stored in sediments) are important in maintaining seagrass populations and their capacity to recover from impacts. Despite their importance, longer-term studies incorporating assessments of seed bank viability are rare and we have little information on how seed banks vary temporally or spatially in their distribution and viability, particularly for tropical and sub-tropical species. This study provides important insights into the seasonal and inter-annual changes in seed bank density and viability, and the implications for local seagrass resilience in Port Curtis and Rodds Bay. The results add substantially to the current knowledge of seagrass seed bank dynamics.

The report details findings from a three year study examining seed bank dynamics for the seagrass *Zostera muelleri* subsp. *capricorni* in Port Curtis and Rodds Bay. Results show that a *Z. muelleri* seed bank was generally present across all sites and times examined, however, temporal trends in seed bank density differed between sites and did not always follow expected seasonal patterns. In addition, viability assessments found that the available seed bank was substantially lower than total seed numbers would suggest, and at times, some sites had no viable seeds present. Viable seeds tended to be located deeper below the sediment surface potentially making them less available for germination and the proportion of the total seed bank that was viable varied considerably between and within sites and over time.

The study highlights the complexity of seed bank dynamics in Port Curtis and Rodds Bay and demonstrates that quantifying resilience requires moving beyond counting densities of seeds to including temporal measurements of seed bank viability. The results provide evidence for the presence of viable seed banks throughout the three year study that are likely to play an important role in conferring resilience to local seagrass meadows. The presence of viable seeds in meadows where no local seed production had been recorded for several years indicates that dispersal of propagules between meadows and sites was likely to be an important process in shaping Port Curtis and Rodds Bay seed banks.

The maintenance of some viable seeds throughout the study meant Port Curtis *Z. muelleri* meadows were well placed to deal with large-scale impacts and had a pathway to recover from meadow scale losses if these viable seeds were to germinate. However, a substantial decline in viable seeds at the end of the program in 2017 was concerning, particularly if seed banks are not replenished during the 2017 seagrass growing season. This result combined with record lows in area and abundance for the largest *Z. muelleri* meadow in Port Curtis suggests that at the end of the study, levels of resilience for some Port Curtis and Rodds Bay meadows were reduced.

The results of this study provide a strong case for the inclusion of assessments of the seed bank that incorporate viability assessment as part of routine seagrass monitoring programs. The low and variable proportion of total seed numbers that were actually viable show that total seed counts alone, will not necessarily provide a true understanding of the state of seed banks. Quantifying these seed bank dynamics is particularly important to gain a clear picture of seagrass meadow resilience given that losses of seagrasses at meadow or larger scales will be reliant on seeds for their recovery.

1 INTRODUCTION

Seagrasses are a key component of the marine environment in Port Curtis and Rodds Bay forming extensive meadows on the intertidal mud and sand banks that line the coast and islands (Figure 1). At their peak, coastal seagrasses cover an area of more than 7,000 hectares in the region (Thomas et al. 2010) and are known to be an important food resource for dugongs (Sobtzick et al. 2013; Rasheed et al 2017a) and green turtles (Limpus 2008; Limpus et al. 2016). The condition of Port Curtis seagrasses has been monitored at least annually since 2002 (see Rasheed et al. 2017b) and more frequently between 2009 and 2016 in response to potential impacts associated with the Western Basin Dredging and Disposal Project (WBDDP) (see Chartrand et al. 2017). These studies have documented the seasonal and annual fluctuations in seagrass presence and have highlighted the critical role that seeds and seed banks likely play in the maintenance and resilience of Port Curtis' seagrasses.

Physical impacts to seagrasses resulting in loss can occur due to a range of environmental and anthropogenic events including storms and flooding (Preen et al. 1995; Poiner et al. 1989), grazing (Preen 1995), anchor and boat damage (La Manna et al. 2015; Kininmonth et al. 2014; Hallac et al. 2012), shipping accidents (Kenworthy et al. 1993) and dredging (Erftemeijer and Lewis 2006). The potential impacts on seagrasses caused by dredging include the physical removal and/or burial of vegetation and effects of increased turbidity and sedimentation (Erftemeijer and Lewis 2006). In Port Curtis, seagrasses have had to respond to a range of natural and anthropogenic pressures in recent years with many meadows declining and recovering through seasonal cycles as well as due to repeated impacts from floods and weather related events, in conjunction with local developments (Chartrand et al. 2017; Rasheed et al 2017b).

A key aspect in understanding the resilience of seagrass meadows to stressors is their capacity to recover from impacts (Kenworthy et al. 2006). Seagrasses are clonal plants and meadows can recover from declines by vegetative means through the extension of rhizomes (asexual reproduction), and by recruitment from propagules (seeds/sexual reproduction) (Rasheed 2004; 1999; Rollón et al. 1998). Seed banks are formed when flowering plants deposit seeds on or in the sediment (Fenner and Thompson 2005). The potential for seagrass meadows to recover from the seed bank is dependent upon the species present, the availability of propagules (viable seeds), and whether growing conditions favour germination (Jarvis and Moore 2015). The maintenance of a viable seed bank is particularly important to seagrass meadow resilience in the event of large-scale declines where much of the adult population is removed and there are limited opportunities for recovery by asexual means.

While seed banks are often measured as simple counts of the number of seeds in the substratum, the available seed bank is really a function of the proportion of that total that remains viable. A viable seed is defined as an embryo that maintains the physiological capability to germinate given the appropriate cues (Murdoch and Ellis 2000). Viability can be affected by biotic (e.g. seed source, predation) and abiotic (e.g. burial depth, sediment type, temperature, salinity) factors in the surrounding environment (Murdoch and Ellis 2000; Baskin and Baskin 1998; Moore et al. 1993). Burial depth in particular can be limiting, as cues for germination may be present at deeper depths (> 5 cm) which initiate the extension of the hypocotyl, however failure of the hypocotyl to reach the surface results in mortality (Jarvis and Moore 2015; Churchill 1983). Seeds buried at deeper depths require a longer hypocotyl than shallower seeds, resulting in a greater time period prior to the emergence of the seedling cotyledon from the sediment surface. As the cotyledon provides oxygen necessary for seedling survival, delays in the emergence of the cotyledon may result in seedling mortality (Churchill 1983) and reductions in seed bank viability.

The suite of seagrass research and monitoring studies commissioned by Gladstone Ports Corporation (GPC) in 2009 associated with WBDDP included some measures of seagrass seed banks (Bryant et al. 2016a), however these did not include assessments of viability, depth structure and temporal and spatial change in seagrass seed banks. This report details the findings of a three year assessment of the seed banks for the dominant seagrass species in Port Curtis, *Zostera muelleri* subsp. *capricorni* (referred to hereafter as *Z. muelleri*) that

aimed to address some of these gaps and gain a clearer understanding of the seed banks and their role for Port Curtis seagrasses. This work was conducted as part of GPC's Ecosystem Research and Monitoring Program (ERMP). The ERMP is a conditioned requirement of the WBDDP designed to acquire a detailed ecological understanding of the marine environment of Port Curtis and Port Alma. Details of the program can be found at the following link:
http://www.gpcl.com.au/SiteAssets/Environment/GPC_Ecosystem_Research_and_Monitoring_Program_Approved_by_DoE_January_2016.pdf

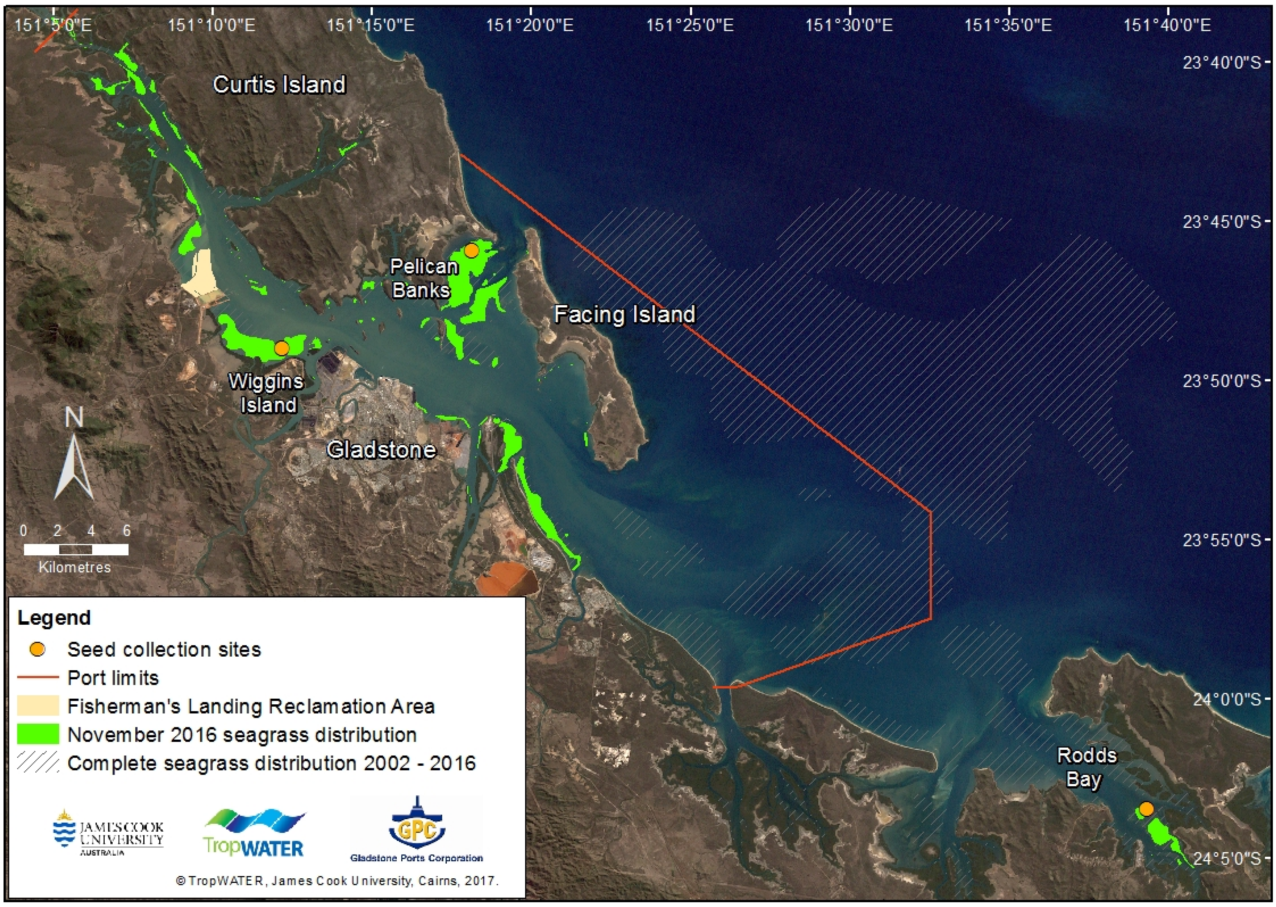


Figure 1: *Zostera muelleri* seed collection sites in Port Curtis and Rodds Bay with seagrass distribution for 2016.

Z. muelleri is known to produce seed banks elsewhere in Queensland including in Moreton Bay to the south of Port Curtis (Conacher et al. 1994a; 1994b). Recent studies to the north, in Cairns, indicate that some viability of *Z. muelleri* seed banks may be retained for more than three years after their likely release from the plant (York & Rasheed 2017). *Z. muelleri* has a specialised flowering shoot called a spathe. The spathes contain male and female flowers that once fertilised, swell to form seeds, and when mature release from the spathe (Figure 2) (Waycott et al. 2004). Flowering and resulting seed production typically occurs in Port Curtis and Rodds Bay between August and February with peak production around November towards the end of the growing season (Bryant et al. 2016a). Those seeds that are incorporated into the seed bank remain until they germinate or are functionally removed due to a loss of viability, or through dispersal or disturbance mechanisms.

1.1 Objectives

The aim of this study was to address critical knowledge gaps related to the density and viability of *Z. muelleri* seed banks in Port Curtis and Rodds Bay. Specifically, the objectives of the project were to:

1. Quantify temporal variation in *Z. muelleri* seed bank density, using the back catalogue of stored seed bank samples collected between 2011 and 2014 and new quarterly samples collected between 2015 and 2017.
2. Monitor changes in the proportion of viable *Z. muelleri* seeds in the seed bank before and after the growing season, and among sediment depths for biannual assessments of seed bank viability conducted each February and May from 2015 to 2017.

Jarvis et al. (2015) reported on the first year of the study including an assessment of the back catalogue of samples as well as results from quarterly (density) and biannual (viability) sampling in 2014/15. Updated findings from quarterly and biannual surveys were reported in 2016 (Bryant et al. 2016b). The current report provides the final results for this project.

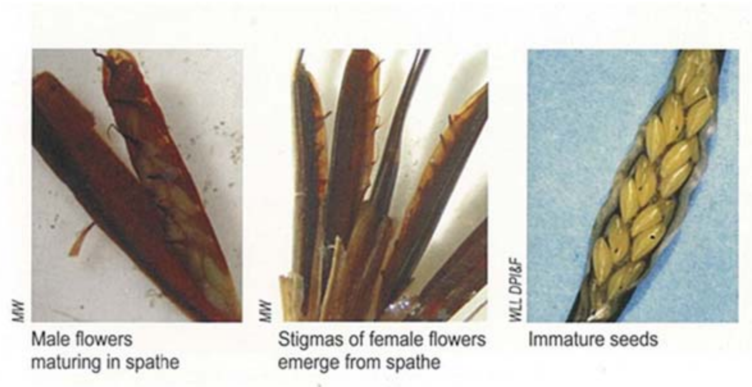


Figure 2. *Zostera muelleri* spathe and seeds (from Waycott et al. 2004)

2 METHODS

2.1 Development of the seed bank density and viability study

This study commenced in 2015 but it builds on the seagrass seed bank assessments that were originally conducted as part of the WBDDP). In 2009, quarterly assessments of seagrass condition at key monitoring meadows throughout Port Curtis and in nearby Rodds Bay were established as a response to the WBDDP. These sites formed the sensitive receptor locations for assessing seagrass condition before, during and after dredging related to the WBDDP. Monitoring was conducted at least quarterly at permanent transect sites between November 2009 and November 2016. Various measures of seagrass condition were made during quarterly assessments including seagrass cover, tissue nutrients, seagrass resilience through reproductive effort and seed banks, and productivity (see Bryant et al. 2016a; Chartrand et al. 2017 for detailed methods and results of the program).

Initially, between 2009–2010, seed banks were assessed at these sites using standard Seagrass-Watch methodology (McKenzie et al. 2000). Sediment cores were sieved in the field using a 1 mm mesh sieve and the contents inspected for *Halodule uninervis* and *Z. muelleri* seeds in the field. These field methods were found to be inadequate to capture seeds of many of the species due to the small size of the seeds, including the dominant *Z. muelleri*. In March 2011, following large scale declines in seagrasses, a more detailed examination of the seed bank was conducted where sediment cores collected at permanent transect sites were taken to the laboratory and sieved to separate various fractions of the sediment, then examined under a dissecting microscope. This method detected numerous *Z. muelleri* seeds in the smaller 710 µm to 1 mm fraction of the sample and seeds of the genus *Halophila* in smaller mesh fractions (McCormack et al. 2013). As a consequence from 2011 onwards cores collected in the field during quarterly sampling events were retained and stored frozen at the TropWATER laboratory for potential future detailed examination of seed banks, that was not possible with field based inspections alone.

For this study a subset of the monitoring sites (Pelican Banks North, Wiggins Island and Rodds Bay (Figure 1)) were selected for detailed processing of samples and new collections for viability assessments (detailed below). These meadows provided a geographical spread relative to dredging associated with the WBDDP and have historically been dominated by *Z. muelleri,* the dominant species in Port Curtis and Rodds Bay. Other seagrass species have ecological significance in the region, but to minimise costs and ensure that information was produced within useful timeframes to inform future management of seagrass resources, we focused our studies on *Z. muelleri* seed banks. The larger seeds of *Z. muelleri* (relative to *Halophila* spp.) are inherently easier to work with and seed core samples could be processed much faster when only a relatively small fraction (710 µm to 1 mm) of the sample needs to be examined. *Z. muelleri* is also one of the more stable and persistent species at these sites (Kilminster et al. 2015).

2.2 Total seed bank density

The sampling methods used followed those established for similar studies in the Port of Cairns (Jarvis et al. 2013). Seagrass seed bank density was assessed quarterly during regular seagrass condition monitoring around February, May, August and November each year. Viability assessments were conducted in February after peak seed release when viable seed densities are expected to be greatest; and in May prior to the onset of the growing season, to test densities of viable seeds (McFarland and Shafer 2011).

Assessments of seed bank density were conducted in conjunction with regular measurements of seagrass condition such as biomass and species composition at the permanent transect sites in February, May, August and November from 2011 to 2016. At each sampling location, sediment cores (50mm in diameter and 100 mm in depth) were collected adjacent to transects at 0 m, 10 m, 20 m, 30 m, 40 m and 50 m intervals (n = 6 per transect; 18 total). Cores were divided into three sections based on sediment depth (0–20 mm, 20–50 mm and

50–100 mm). Samples were stored on ice for transport to the TropWATER laboratory in Cairns, and stored at -20°C until processing.

Sediment cores collected in February and May from 2015 to 2017, were also used to assess seed viability (Section 2.2). These cores were stored on ice for transport to the TropWATER laboratory in Cairns and stored at 8–10°C for processing, which occurred within one week of collection (Marion and Orth 2010).

All sediment samples were wet sieved with fresh water and the 710 µm to 1 mm fraction of the sediment was inspected for *Z. muelleri* seeds using a dissecting microscope (Figure 3). The greater than 1 mm fraction was also assessed in case any larger seeds were present, but for *Z. muelleri,* all seeds were found in the smaller 710 µm to 1 mm fraction. All seeds were identified, photographed and catalogued for the TropWATER seed bank library. The remaining fraction of the sediment was frozen and stored. Seed density data were reported as the mean ± standard error (SE) total number of seeds m^{-2} $site^{-1}$.

Figure 3. Processing of sediment seed bank samples.

2.3 Seed bank viability

Seed bank viability assessments were conducted in February and May of 2015, 2016 and 2017. All seed samples were tested for viability within one week of collection using tetrazolium chloride (Sawma and Mohler 2002; Conacher et al. 1994a; Lakon 1949). Seed embryos were removed from their seed coats and soaked in a 0.5% tetrazolium chloride solution for 48 hours before examination under a dissecting microscope at 10x magnification (Conacher et al. 1994a). Seeds with a red (pink to brown) stained axial hypocotyl were counted as viable (Conacher et al. 1994a; Harrison 1993; Taylor 1957) (Figure 4). Viability data are reported as the mean ± standard error of the percentage of viable seeds relative to total seeds (viable + non-viable).

Figure 4. Examples of stained viable and non-viable *Zostera muelleri* seeds using tetrazolium chloride.

2.4 Data analysis

Total Seed Bank Density

Analysis of total seed bank density (viable + non-viable seeds per core) was conducted independently for each site (Rodds Bay, Pelican Banks North, Wiggins Island) using generalised linear mixed effects models (GLMM). Data exploration protocols prior to all analyses followed Zuur et al. (2010) and included checks for collinearity and zero inflation. Overdispersion was a problem in most preliminary model runs using the poisson distribution for count data; where required this was corrected with the use of a negative binomial distribution. We used the *glmmADMB* package in R version 3.3.1 (Skaug et al. 2016; Fournier et al.2012) for all seed bank density analyses.

For each analysis, a global model was compared against model subsets including the null hypothesis (see Appendices A to C). The random effect of distance nested in transect was included in each model. The best-fit model was considered the simplest model with the lowest Akaike's Information Criterion (AIC) that fell within two of the lowest AIC (Burnham and Anderson 2002), and where residual plots did not demonstrate any deviations from homoscedasticity or normality. Statistical significance of factors in the best-fit models were tested using the Wald chi-square statistic in the *car* package in R (Weisberg 2011).

1. Temporal Effects on Total Seed Bank Density - Total seed bank density was compared among all years and sampling months using negative binomial GLMMs. All sites were sampled quarterly: Rodds Bay from August 2011 to May 2017, and Pelican Banks North and Wiggins Island from March 2011–May 2017. At Wiggins Island, seed bank data were not collected in 2013 due to restrictions in site access, and February and May 2014 data were excluded from statistical analysis due to differences in sampling methods. Five candidate models were compared for each site using the AIC, where the global model was *seeds ~ month + year + (1|transect: distance)*, and *seeds ~ date + (1|transect:distance)* was a proxy for a *month*year* interaction because not all months in each year were sampled.

2. Seasonal Effects on Total Seed Bank Density - Total seed bank density was compared among years and seasons using negative binomial GLMMs. The seasons were directly following the growing season (February) and towards the end of the senescent season (May). Five candidate models were compared for each site using the AIC, where the global model was *seeds ~ season*year + (1|transect: distance)*. Analysis was limited to years where data were collected in February and May: Pelican Banks North and Rodds Bay were analysed from 2012–2017 and Wiggins Island was analysed for 2012 and 2015–2017.

3. Sediment Depth and Temporal Effects on Total Seed Bank Density - Total seed bank density was compared among all years, sampling months, and sediment depths (0–20 mm, 20–50 mm, 50–100 mm) using a poisson GLMM for Rodds Bay and Wiggins Island, and a negative binomial GLMM for Pelican Banks North. Rodds Bay and Pelican Banks North were sampled quarterly and sectioned for depth from November 2013–May 2017, and Wiggins Island from August 2014–May 2017). Nine candidate models were compared for each site using the AIC, where the global model was *seeds ~ month + year + (1|transect: distance)*, and seeds ~ *date + depth + (1|transect: distance)* was a proxy for a *month*year* interaction because not all months in each year were sampled.

Seed Bank Viability

Analysis of seed viability (proportion of viable seeds, *pvs*) was conducted independently for each site (Rodds Bay, Pelican Banks North, Wiggins Island). We used zero/one inflated beta (ZOIB) regression models, which model response variables bound between or equal to 0 or 1, including percent and proportional data. Data exploration protocols prior to all analyses followed Zuur et al. (2010). We used the *gamlss* package for all seed bank viability analysis (Rigby and Stasinopoulos 2005). Likelihood ratio tests (LRT) were run to test the significance of explanatory terms in the models. Pelican Banks data could not be analysed due to the small sample size.

1. Seasonal Effects on Seed Bank Viability - Seasonal changes in proportion of viable seeds were compared directly following the growing season (February), then towards the end of the senescent season (May), and among years (2015–2017) at Rodds Bay and Wiggins Island. The global model for each site was *pvs ~ season*year*. Model selection was performed on each global model using the 'drop1' function (Chambers 1992) in R.

2. Sediment Depth and Temporal Effects on Seed Bank Viability - The effect of sediment depth (0–20 mm, 20–50 mm, 50–100 mm), season (growing and senescent) and year (2015–2017) on the proportion of viable seeds was analysed for Rodds Bay and Wiggins Island. The global model for each site was *pvs ~ depth*season*year*. Model selection was performed on each global model by stepwise selection using the 'step' function in R.

3 RESULTS

Seed bank examinations revealed a *Z. muelleri* seed bank was present at all sites for every time sampled, however the seasonality and inter-annual change in seed abundance varied between sites and times. While seeds were present they were not always viable and for some sites and times this resulted in the absence of a viable seed bank. There was a general trend for viable seeds to be found at deeper depths within the sediment cores and an overall decline in the seed bank across all sites during 2016 and 2017.

3.1 Long term changes in total seed bank density

Seed bank density generally declined over time at all sites, despite varying degrees of temporal variation (Table 1). At Pelican Banks North, seed bank density varied significantly among years (Table 1). Seed bank density peaked in 2011 (773 $\pm$ 112 seeds m^{-2}) and was lowest in 2017 (57 $\pm$ 34 seeds m^{-2}) (Figure 4a). At Rodds Bay, the decline in seed bank density was more temporally variable, with significant variation among sampling dates (Table 1). The greatest seed bank densities (>1300 seeds m^{-2}) were recorded earlier in the study (August and November 2011, May 2012), and the lowest densities (<200 seeds m^{-2}) were recorded later in the study (August 2013, February and May 2017) (Figure 4b). At Wiggins Island, seed bank density varied significantly annually and among sampling months (Table 1). Seed bank density declined from a peak of 821 ± 181 seeds m^{-2} in 2014 to just 85 ± 38 seeds m^{-2} in 2017 (Figure 4). Seed bank density halved between the general May peak (603 ± 108 seeds m^{-2}, all years) and August decline (300 ± 56 seeds m^{-2}, all years).

Table 1. Results for the GLMMs for the effect of month and year on seed density. Coefficients table for best fit models is in Appendix B.
Date was used as a proxy for month*year interaction because not all months were sampled each year. Significant effects in **bold**.

Site	Model	df	Wald X^2	p-value
Pelican Banks North	Intercept	1	3.87	<0.05
	Year	**7**	**202.23**	**<0.001**
Rodds Bay	Intercept	1	15.08	<0.001
	Date	**23**	**486.83**	**<0.001**
Wiggins Island	**Intercept**	**1**	**2.81**	**0.09**
	Month	**3**	**19.79**	**<0.001**
	Year	**5**	**65.26**	**<0.001**

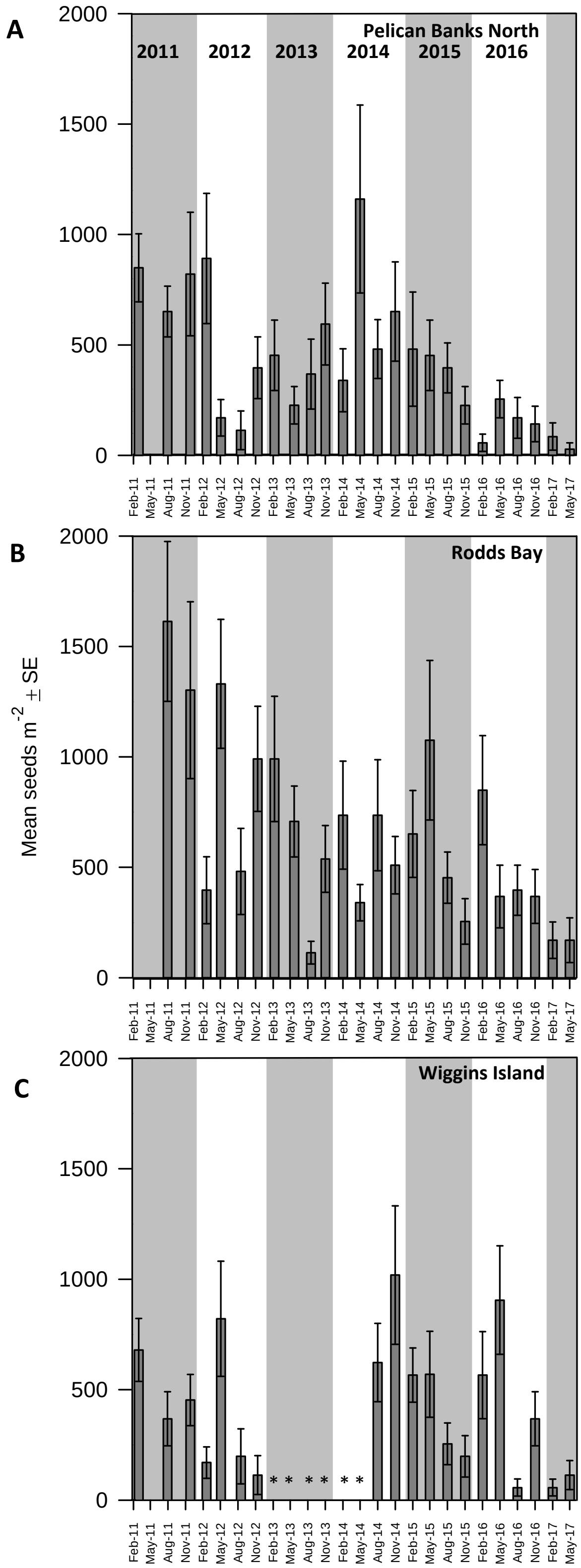


Figure 4. Mean seed bank density (± SE) at (A) Pelican Banks North, (B) Rodds Bay and (C) Wiggins Island from February 2011 until May 2017. * Wiggins Island data were not collected in 2013 due to access issues associated with port development preventing sampling , and February and May 2014 due to sampling methods.

At Pelican Banks North, peaks in spathe production and seeds found in the sediment seed bank (Figure 5) generally followed peaks in seagrass percent cover. After the seagrass growing season (November), when seagrass cover is at its highest it is expected that spathe production and seed release following this period will also be at its highest (February). The exception to this pattern is in 2013/2014 and 2016/2017 where there were no spathes found (Figure 5). Further to this since 2015 there has been a gradual decline in seagrass percent cover, coinciding with spathe and seed production declines.

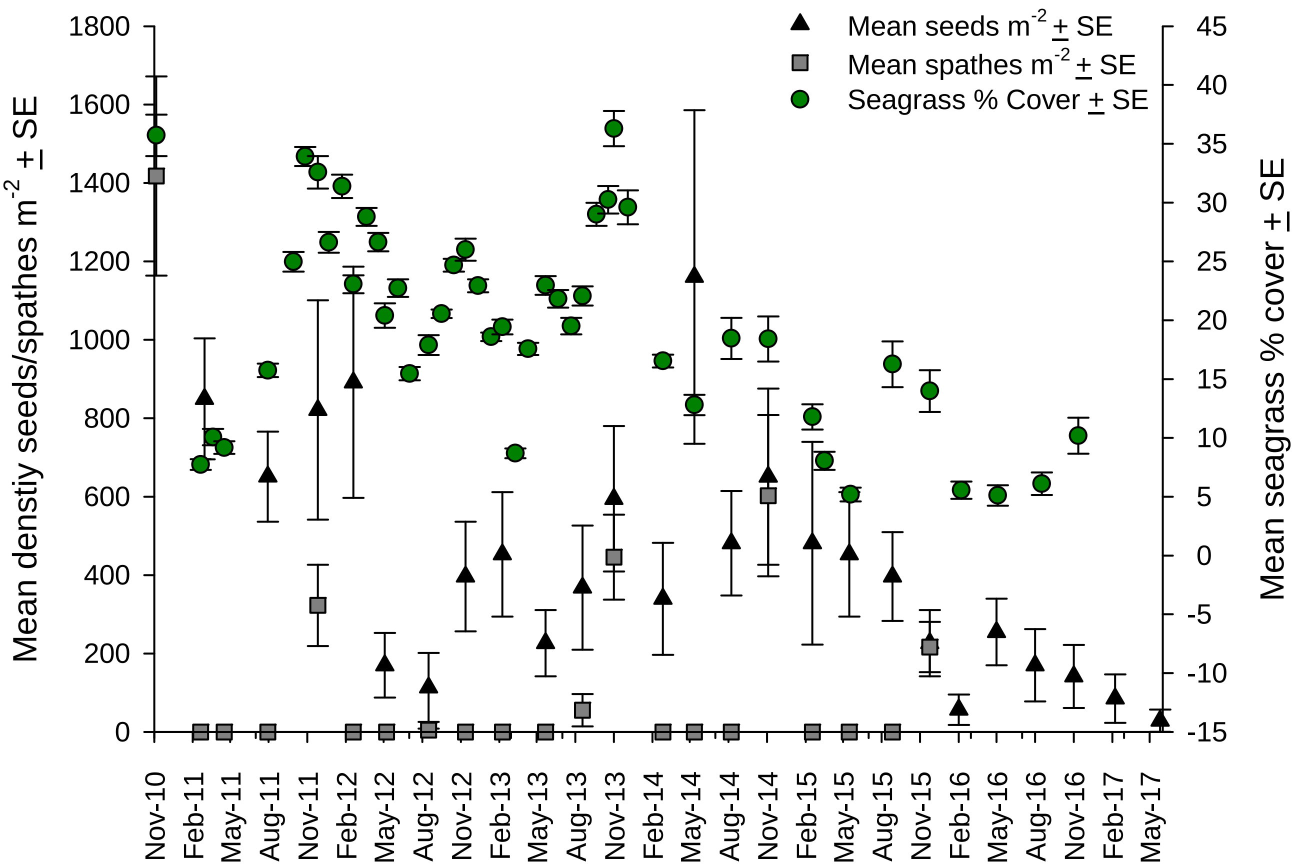


Figure 5. Mean (+ SE) seagrass percent cover and spathes m^{-2} at the annual peak in the seagrass growing season (November), and mean seed bank density, following expected periods of peak seed production at Pelican Banks North, November 2010 to May 2017.

3.2 Seasonal changes in total seed bank density

Seed bank density varied significantly among seasons and years at Wiggins Island and Rodds Bay (Table 2). At Wiggins Island, seed bank density during the senescent season (602 ± 108 seeds m^{-2}) was almost double the density than during the growing season (340 ± 66 seeds m^{-2}). In Rodds Bay, seed bank densities had no clear seasonality; density was greater in the growing season than the senescent season in 2013, 2014, and 2016, in 2012 and 2015 the seasonal patterns was reversed, and in 2017 there was no difference among seasons (Figure 6: Table 2). There was no seasonal variation among seed bank density at Pelican Banks North (Figure 6; Table 2).

Table 2. Results for the GLMMs for the effect of season and year on seed bank density. Coefficients table for best fit models is in Appendix D.
Significant effects in **bold**.

Site	Model	df	Wald X^2	p-value
Pelican Banks North	Intercept	1	1.23	0.27
	Year	**5**	**19.54**	**<0.01**
Rodds Bay	Intercept	1	1.88	0.17
	Season	1	9.54	<0.01
	Year	5	10.87	0.05
	Season x Year	**5**	**42.71**	**<0.001**
Wiggins Island	Intercept	1	2.62	0.11
	Season	**1**	**5.70**	**<0.05**
	Year	**3**	**11.56**	**<0.01**

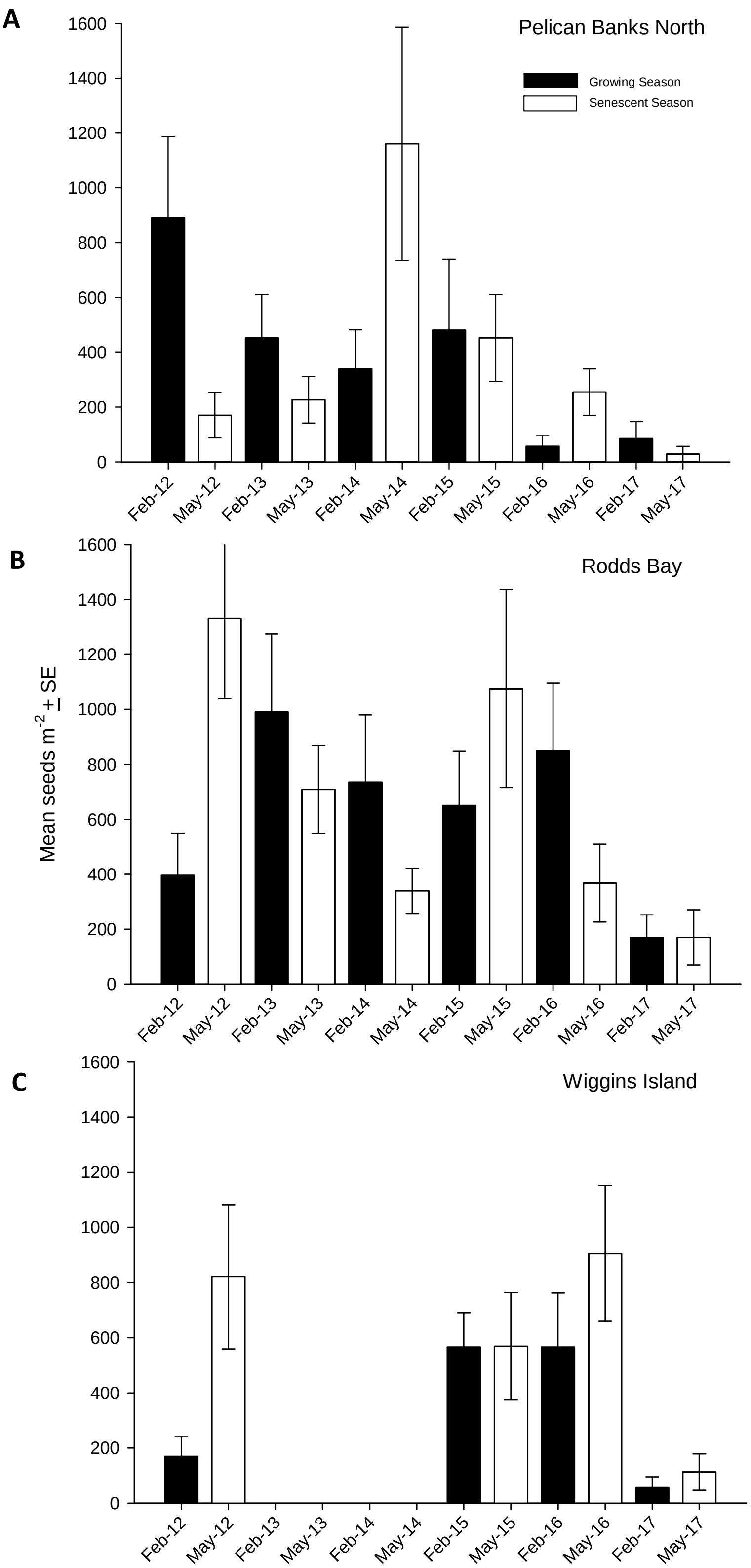


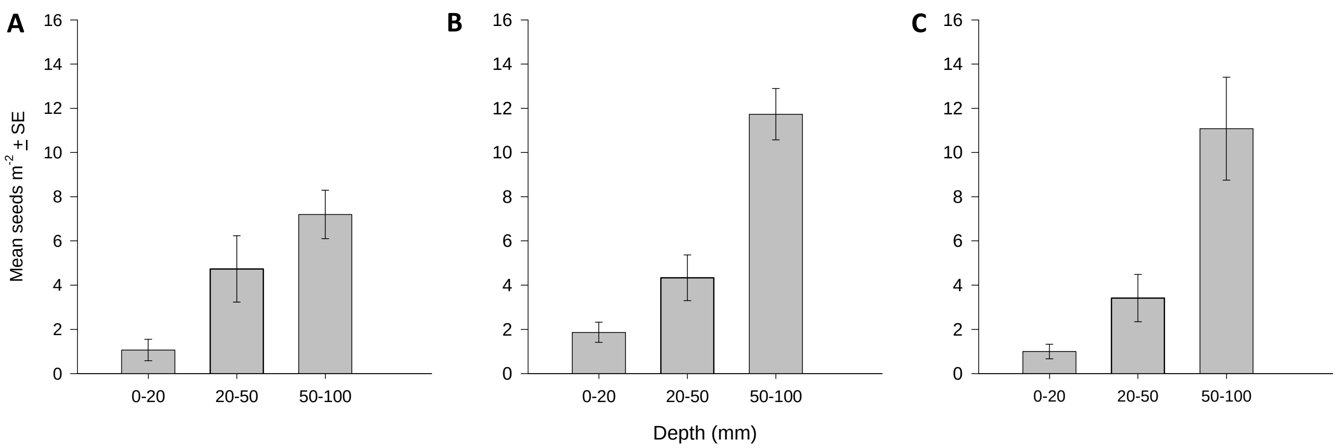
Figure 6. Average seed bank density (mean seeds m^{-2} ± SE) at (A) Pelican Banks North, (B) Rodds Bay and (C) Wiggins Island showing seed bank density at the end of the growing season (black) which is the period of maximum seed production (February) and during the senescent season (white)(May). Data were not collected at Wiggins Island in 2013 and February and May 2014 data were not included in statistical analysis.

3.3 Seed bank density and sediment depth

Seed bank density increased significantly with increasing sediment depth at all sites. Pelican Banks North had 55% of its seeds found at depths deeper than 50 mm, 65 % at Rodds Bay and 72% at Wiggins Island (Figure 7; Table 3).

Table 3. Results for the poisson (Wiggins Island and Rodds Bay) and negative binomial (Pelican Banks North) GLMMs for the effect of depth, month and year on seed density. Coefficients table for best fit models is in Appendix F. Significant effects in **bold**.

Site	Model	df	Wald X^2	p-value
Pelican Banks North	**Intercept**	**1**	**48.21**	**<0.001**
	Depth	**2**	**49.51**	**<0.001**
Rodds Bay	**Intercept**	**1**	**62.69**	**<0.001**
	Depth	**2**	**108.97**	**<0.001**
Wiggins Island	**Intercept**	**1**	**48.18**	**<0.001**
	Depth	**2**	**94.24**	**<0.001**



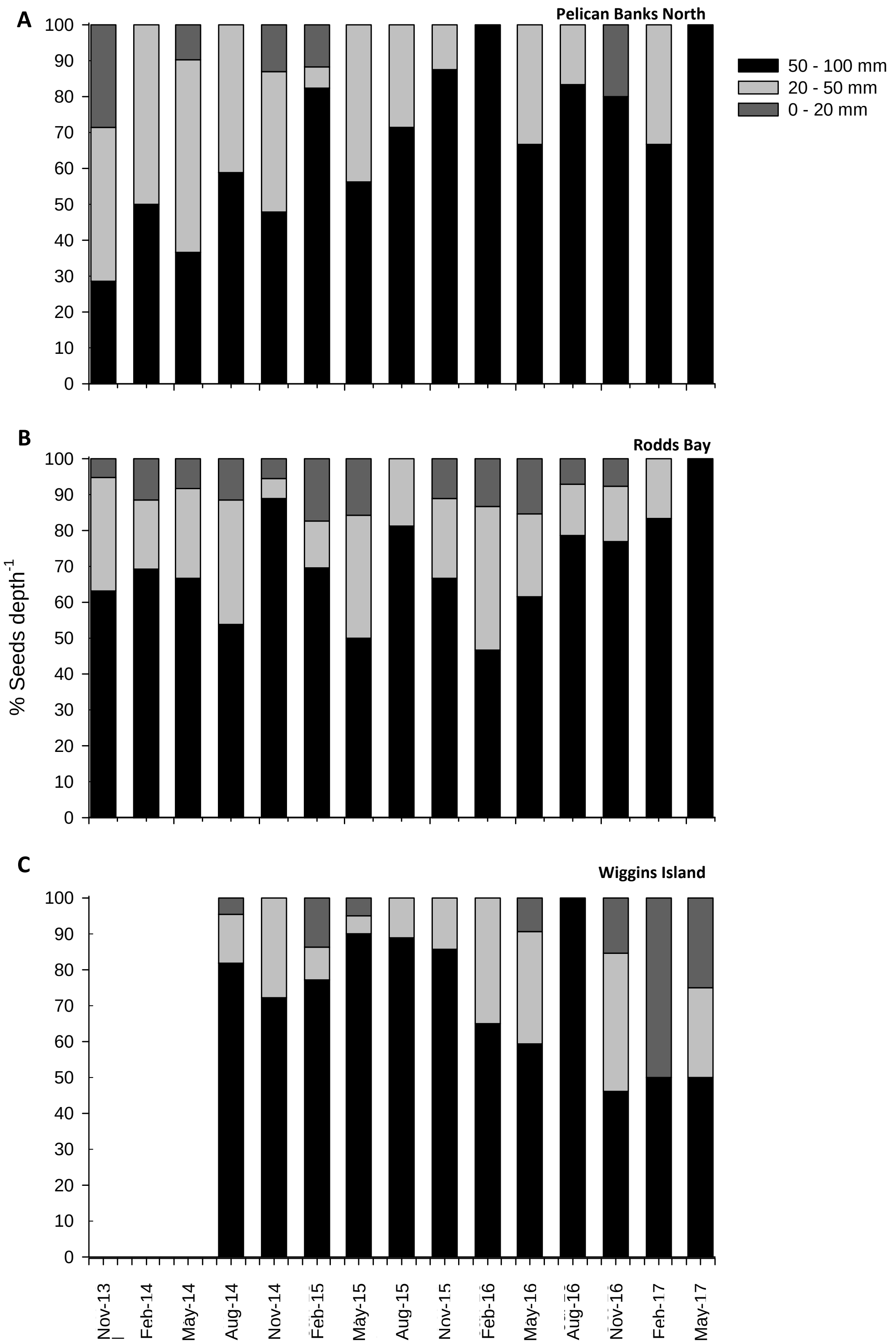


Figure 7. Depth distribution of seagrass seeds expressed as the percent of total seeds at 0–20 mm, 20–50 mm and 50–100 mm depths for (A) Pelican Banks, (B) Rodds Bay and (C) Wiggins Island.

3.4 Seed bank viability

At Rodds Bay, the proportion of viable seeds was influenced by a significant interaction between season and year (LRT = 7.04, *df* = 2, *P* <0.05). The proportion of viable seeds was greater in the growing season (February) than the senescent season (May) in 2015, but greater in the senescent season in 2016 and 2017. Seed viability was greater in 2017 for both seasons than in 2015 and 2016 (Figure 8). At Wiggins Island, the proportion of viable seeds declined significantly between 2015 and 2017 (LRT = 7.64, *df* = 2, *P* <0.05) and was significantly higher in the growing season than senescent season (LRT = 6.15, *df* = 1, *P* <0.05) (Figure 8). The interaction between season and year was not significant (LRT = 7.80, *df* = 5, *P* = 0.17). Wiggins Island was characterised as having the lowest proportion (9.33% ± SE) of viable seeds among sites. Coefficients table for best fit models is in Appendix G. At Pelican Banks North, the low number of seeds found did not allow for statistical analysis of seed viability; however the seeds collected in 2016 and 2017 (both seasons) were all viable (Figure 8).

Analysis of the proportion of viable seeds at depth showed no significant results due to the low number of seeds found. However the proportion of viable seeds was greatest at depths of 50–100 mm across all sites (Figure 9). At Pelican Banks North most viable seeds were recorded at 50–100 mm sediment depth and no viable seeds were found in 0–20 mm depth (Figure 9). At Rodds Bay, most viable seeds were found at depths from 20–100 mm, with only a few found in the 0–20 mm depth profile (Figure 9). At Wiggins Island, while a few viable seeds were found in the 0–20 mm depth profile, most were found in the deeper sediment at 50–100 mm, and no viable seeds were found after May 2016.

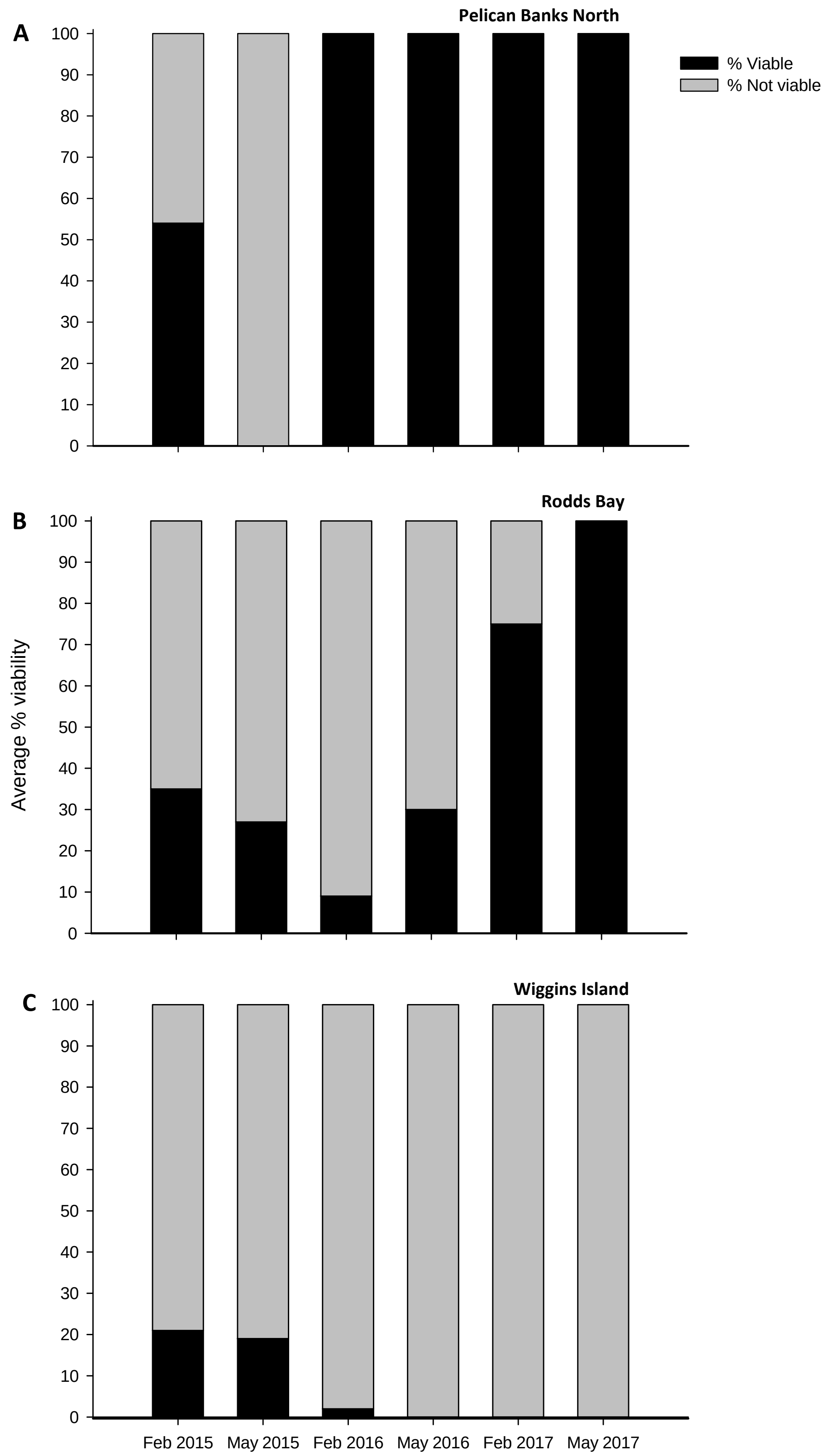


Figure 8. Average percentage of viable seeds (black) and non-viable (grey) in the seed bank at (A) Pelican Banks North, (B) Rodds Bay and (C) Wiggins Island following the period of maximum seed production during the seagrass growing period (February) and during the senescent season (May).

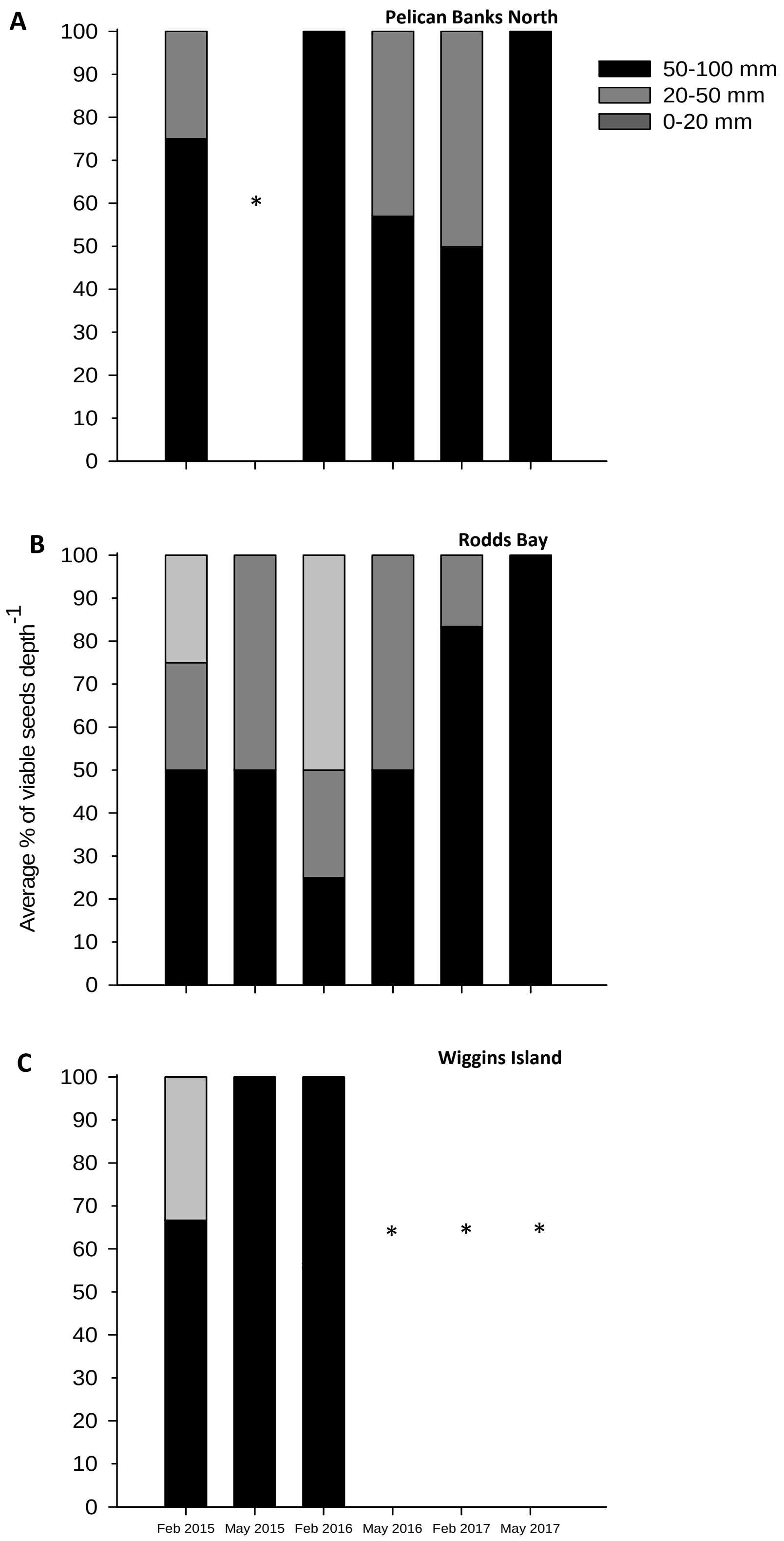


Figure 9. Mean proportion of viable *Z. muelleri* seeds at 0–20 mm (light grey), 20–50 mm (dark grey) and 50–100 mm (black) depths for (A) Pelican Banks North, (B) Rodds Bay and (C) Wiggins Island. * represents no viable seeds collected.

4 DISCUSSION

Despite the importance of seagrass seeds and seed banks for maintaining seagrass populations and their capacity to recover from impacts, longer-term studies, incorporating assessments of seed bank viability are rare. This study provides an important insight into the seasonal and inter-annual changes in seed bank density and viability, and the implications this may have for local seagrass resilience in Port Curtis. Results show that a *Z. muelleri* seed bank is generally present across all sites and times examined, however, temporal trends in seed bank density differed between sites and did not always follow expected seasonal patterns. In addition, viability assessments found that the available seed bank was substantially lower than total seed numbers would suggest, and at times, some sites had no viable seeds present. Viable seeds tended to be located deeper below the sediment surface, potentially making them less available for germination. Of most concern was the recent decline in seeds across all sites to their lowest recorded level in 2017. Despite some increases in the proportion of seeds that were viable, this still resulted in a large reduction to the actual total number of viable seeds that would be available to initiate recovery. A trend that if continued may leave the meadows less able to recover from large scale losses that rely on seeds.

Seed density found in Port Curtis and Rodds Bay meadows over the course of this study was generally higher than that recorded for similar populations of *Z. muelleri* in tropical Queensland such as described in Cairns and Mourilyan Harbour (Reason et al. 2017; York and Rasheed 2017) and on a par with assessments to the south in Moreton Bay (Conacher et al. 1994b). However, Cairns and Mourilyan meadows had suffered almost complete loss of seagrass by 2011 (McKenna et al. 2015) and it is likely the low seed bank densities at these locations were a reflection of a lack of recent replenishment and not necessarily representative of a seed-bank under more typical circumstances. Regardless, the results in Port Curtis indicated that meadows maintained a viable seed bank during the course of our study with the possible exception of 2017 for some meadows.

The precise timing of seed release in Port Curtis and Rodds Bay is not well understood but is likely controlled by a range of environmental factors, such as water temperature, depth, and day length (Conacher et al. 1994b). At Pelican Banks North, where we have the longest history of quarterly measurements (2009–2017), peaks in reproductive spathe density occurred in November each year (Chartrand et al. 2017). Flowering is thought to last up to four months over the seagrass growing season (from August to November) but it is possible that flowering extends beyond this period. Given the absence of spathes by each February in this study, it is likely that maximum seed release and replenishment of the seed bank occurs towards the end of the growing season (November to January) following peaks in spathe density. At two of our sites, Rodds Bay and Wiggins Island, *Z. muelleri* spathes were not detected growing locally during routine monitoring since November 2009 and September 2012 respectively (Chartrand et al. 2017). It is unlikely that seeds found at these sites could remain intact and viable for periods longer than 3 years after production based on Conacher et al. (1994a) and unpublished data collected at other Queensland sites (JCU unpublished data from Cairns). Therefore seeds found at these sites are likely to be from dispersal of sexual propagules from other nearby donor meadows.

Based on similar studies (Conacher et al. 1994b) and local spathe density data (Chartrand et al. 2017), we expected to find higher seed densities within the seed bank in summer (February) immediately following periods of maximum seed production. At Pelican Banks North, seed bank densities followed these expected trends for some years but in other years and at other sites trends were less clear. We also expected that seed bank density would remain stable or decline over the seagrass senescent period (from February to May) due to mortality, predation or the 'secondary dispersal' of seeds through biotic or abiotic mechanisms (Kendrick et al. 2012; Orth et al. 2006). However, seasonal analysis often revealed significant increases in seed density over this senescent period, particularly at Wiggins Island where seed bank densities were consistently higher in May than in February (all years except 2015), and also at Rodds Bay (2012 and 2015) and Pelican Banks (2014 and 2016). This indicates that secondary dispersal of seeds may be occurring from other locations after the production of new seeds has ceased. It is also possible that spathes and seed material may take some time to be incorporated into the sediment particularly when they have been dispersed to a site from other locations.

Connectivity between seagrass meadows via dispersal of propagules, including seeds, is essential for maintaining resilience of meadows to large-scale disturbance (Kendrick et al. 2012). *Zostera* spp. seeds are negatively buoyant with dispersal distances of only a few meters (Orth et al. 1994). However, several mechanisms for the wider dispersal of sexual propagules are described in the literature. Vegetative fragments dislodged by natural processes such as wave action, storm events and grazing herbivores or anthropogenic disturbances such as dredging and anchor or propeller scarring have the ability to carry seeds and reproductive structures distances of 100's of kilometres (Grech et al. 2016; Stafford-Bell et al. 2015; McMahon et al. 2014). The spathes containing fruits of *Z. muelleri* remain buoyant for some time once broken off from the parent plant and are commonly seen as rafts of material that offer a mechanism for longer distance dispersal (Stafford-Bell et al. 2015; Källström et al. 2008). Vegetative fragments of *Z. muelleri* in Port Curtis have recently been shown to remain buoyant for an average of three weeks (Weatherall et al. 2016) and similar studies have shown prolonged buoyancy up to five weeks (Stafford-Bell et al. 2015) providing ample time for dispersal to nearby meadows and explanations for the delayed incorporation into the sediment seed bank.

Seeds can also be dispersed via the consumption of plant reproductive material by marine herbivores. Both green turtle and dugong feeding activity have been known to break off fragments resulting in spathes floating out to propagate elsewhere. Dugongs and turtles in tropical Queensland have been reported to disperse significant numbers of viable *Z. muelleri* seeds via consumption and excretion over possible distances of more than 600 km (Tol et al. 2017). In Port Curtis and Rodds Bay, dugong foraging is well documented at all of our seed bank study sites (Davies et al. 2016; Rasheed et al. 2017a) and may play a significant role in the dispersal of seeds between meadows. At Rodds Bay, dugong and turtle feeding activity recorded at meadows near the mouth of the bay could provide a dispersal mechanism for seeds to the meadows further inside the bay (Rasheed et al. 2017a) where our seed bank monitoring site was located. This could explain the continued presence of a seed bank at the sampling site despite the decrease in seagrass area and biomass over the past two years and the absence of local reproductive spathes since 2009. It is also possible that dispersal of propagules into the area occurs through more passive means including wave, wind and current action transporting floating propagules.

Secondary dispersal of seeds can also occur through the movement of seeds within the seed bank after the initial period of incorporation (Kendrick et al. 2012; Orth et al. 2006). Bioturbation by animals living in the sediment (Townsend and Fonesca 1998) as well as wind and storm activity (Hammerstrom et al. 2006) can move seeds both horizontally and vertically within the sediment and may explain increases in seed bank densities outside of periods of maximum seed production.

Burial depth of seagrass seeds can have a limiting effect on the resilience provided by the seed bank (Jarvis and Moore 2015). Seeds that are buried too deeply are effectively removed from the viable seed bank as they are not capable of germinating and successfully producing seedlings (Jarvis and Moore 2015; Granger et al. 2000). While the effects of burial depth on the viability and germination of *Z. muelleri* are unknown, germination of the similar species *Z. marina* is limited when seeds are buried deeper than 50 mm (Jarvis and Moore 2015). Sedimentation dynamics at Pelican Banks was studied by Benham et al. (2016) over 2014 and 2015 and indicates an increase in sediment load following the wet season which resulted in a significant reduction of *Z. muelleri* rhizome growth rates at depths of ≥10 mm (Benham et al. 2016). These findings coincide with our field observations (in February and May 2016/17) of changes at the site including an increase in areas of bare sandy sediment and the burial of seagrasses. In Port Curtis and Rodds Bay, more than 60% of seeds were found at depths deeper than 50 mm during most sampling events. At Pelican Banks North, there was an increasing trend in the proportion of seeds found deeper than 50 mm over the sampling years. It is possible that viable seeds in the shallow depths had already germinated therefore leaving only viable seeds in the deeper sediment profile. The resilience to disturbance and capacity for a seagrass meadow to recover following a large scale impact will be directly influenced by factors affecting germination and seedling success (Jarvis and Moore 2015). If germination success for *Z. muelleri* seeds suffers from similar inhibition with burial depth as other species in the genus that have been studied (Jarvis and Moore 2015) then this is likely to limit the effectiveness of the sediment seed bank for Port Curtis and Rodds Bay seagrass meadows.

Results of viability assessments undertaken biannually (February and May) emphasise the importance of understanding seed bank viability and not just total seed bank numbers, with the viable proportion of the seed bank variable both within and between sites and times. From 2015 to 2017, the Pelican Banks North site had the greatest proportion of viable seeds, followed by Rodds Bay then Wiggins Island with the lowest. In 2017, a relatively high proportion of seeds remained viable at both Pelican Banks North and Rodds Bay compared with previous years. However, despite a high proportion being viable, the total number of seeds at Pelican Banks North was the lowest on record, meaning the effective seed bank available for germination had actually declined. At Rodds Bay, the increase in the proportion of viable seeds from February to May was somewhat surprising, but provides further evidence of secondary dispersal or that sexual propagules are being supplied from an outside source. The low proportion of viable seeds at Wiggins Island in 2017 means that this meadow will be heavily reliant on the remaining adult population (remaining intact meadow) and or the recruitment of viable fragments or seeds from donor meadows to recover over the coming growing season. These insights are only possible when the total density and viability of seeds are considered together.

The exact longevity of viable seeds in the seed banks in Port Curtis and Rodds Bay is unknown. Decreases in seed germination and viability have been attributed to burial depth, anoxia, sediment type, temperature, predation and mortality (Jarvis and Moore 2015; Sumoski and Orth 2012; Fishman and Orth 1996; Conacher et al. 1994a; Moore et al. 1993). Further investigation into the change in seed viability over time (persistence) by directly following a cohort of seeds of a known age would be required to quantify this specifically for Port Curtis and Rodds Bay. However, evidence from other locations in Queensland would suggest that some viability of *Z. muelleri* seeds is maintained for at around two years after seed production (York and Rasheed 2017).

The high variance recorded in both the total numbers of seeds and seed viability in this study may be typical for many seed banks. Previous studies have found seed banks to be naturally patchy and as such taking many smaller samples is recommended to reduce variation (McFarland and Shafer 2011). In this study, we were limited by the number of samples we could process in the timeframe dictated by the viability sampling methodology (samples collected and processed completely within one week of sampling). Additional samples taken at each site would be expected to reduce variation in the data, however this would come at a substantial logistical cost and expense. Establishing the spatial structure of the seed bank across the meadow rather than focusing work at the smaller permanent transect sites may assist in reducing variability in results, but this was outside the scope of the current study.

5 CONCLUSION & RECOMMENDATIONS

In highly disturbed seagrass meadows, a period of seed dormancy is an obvious advantage, enabling the regeneration of seagrasses even where all adult plant material has been lost (Rasheed 2004). Recent recovery of *Z. muelleri* meadows from near complete loss in Cairns, for example, was only possible through the germination of remaining viable seeds in the local seed bank (York and Rasheed 2017). Yet nearby in Mourilyan Harbour, where similar meadow loss occurred, recovery has not been possible as no seed bank remained (Reason et al. 2017). It is likely then, that the seed banks in Port Curtis and Rodds Bay described in our study play an important role in conferring resilience to local seagrass meadows.

Final results of the three year study highlight the complexity of seed bank dynamics in Port Curtis and Rodds Bay and demonstrate that quantifying resilience requires moving beyond counting densities of seeds to including temporal measurements of seed bank viability and ideally germination success. The results provide evidence for the presence of a viable seed bank. However, the marked decline of the seed bank during the final sampling period in 2017 is concerning, particularly if seed banks are not replenished during the 2017 growing season. With the results of the most recent monitoring of seagrass meadows in Port Curtis showing record lows in area and abundance for the Pelican Banks *Z. muelleri* meadow, the continued health of the seed bank is particularly important. Understanding how seed banks interact with sediment dynamics, germination cues and the adult meadow to confer resilience to seagrasses is a key question and the results of this study provide the foundation for continuing this work.

This study highlights the importance of understanding seed bank dynamics including viability as part of seagrass monitoring programs that aim to understand seagrass meadow resilience. We recognise that this may not always be possible or practical as many species have small seeds that are difficult to work with, such as those from the genus *Halophila* and assessments and analysis would be expensive. However, for species with larger seeds such as *Z. muelleri* and *H. uninervis* that form the foundation of many coastal meadows in Queensland, the methods developed in this project could be applied to at least measure the change in the status of viable seed banks and with further work how this may act to confer resilience to meadows.

This work has significantly advanced our understanding of seed bank dynamics and provides a good foundation for further studies that would assist in understanding seagrass resilience and seed bank dynamics including:

1. Broader (meadow wide) assessments of the seed bank to establish spatial seed bank structures.
2. Manipulative field studies examining the persistence of *Z. muelleri* seed banks, i.e. changes in viability over time (see Jarvis and Moore 2015).
3. Studies examining the environmental cues for germination and rates of germination and seedling success.
4. Examinations of seed dispersal mechanisms to assess likely sink and source meadows.
5. Greater understanding of the role of burial depth and sediment dynamics in determining how viable seeds confer resilience to meadows

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7 APPENDICES

A) Comparison of negative binomial models for the effect of month and year, and date as a proxy for month*year interaction, on total seed density. Random effect is distance nested within transect. The best fit model selected for each site is in **bold**.

Site	Candidate models	df	AIC
Wiggins Island	**M4 = seeds ~month + year + (1|transect:distance)**	**11**	**818.45**
	M5 = seeds ~ date + (1|transect:distance)	21	825.30
	M2 = seeds ~ year + (1|transect:distance)	8	844.96
	M3 = seeds ~ month + (1|transect:distance)	6	860.75
	M1 = seeds ~ 1 + (1|transect:distance)	3	861.78
Rodds Bay	**M5 = seeds ~ date + (1|transect:distance)**	**26**	**1241.43**
	M4 = seeds ~ month + year + (1|transect:distance)	12	1253.96
	M2 = seeds ~ year + (1|transect:distance)	9	1259.10
	M1 = seeds ~ 1 + (1|transect:distance)	3	1303.63
	M3 = seeds ~ month + (1|transect:distance)	6	1309.44
Pelican Banks North	**M2 = seeds ~ year + (1|transect:distance)**	**9**	**1034.83**
	M4 = seeds ~ month + year + (1|transect:distance)	12	1038.82
	M5 = seeds ~ date + (1|transect:distance)	27	1045.90
	M1 = seeds ~ 1 + (1|transect:distance)	3	1078.96
	M3 = seeds ~ month + (1|transect:distance)	6	1081.93

B) Coefficients of best fit models for the effect of month, year or date, on total seed density at Wiggins Island, Rodds Bay, and Pelican Banks North. Significance codes for p-values: "***" = 0.001; "**" = 0.01; "*" = 0.05; "." = 0.1; " " = 1.

Site		Estimate	SE	z-value	p-value
Wiggins Island	(Intercept)	0.40	0.24	1.68	.
	Month_May	0.66	0.25	2.68	**
	Month_August	-0.85	0.26	-3.30	***
	Month_November	-0.53	0.25	-2.16	*
	Year_2012	-0.87	0.29	-2.97	**
	Year_2014	0.75	0.30	2.51	*
	Year_2015	-0.57	0.28	-2.07	*
	Year_2016	-0.47	0.27	-1.72	.
	Year_2017	-2.57	0.50	-5.08	***
Rodds Bay	(Intercept)	**1.12**	**0.29**	**3.88**	***
	Date_Nov.2011	-0.27	0.329	-0.83	
	Date_Feb.2012	-1.62	0.40	-4.07	***
	Date May.2012	-0.43	0.35	-1.32	
	Date Aug.2012	-1.45	0.38	-3.79	***
	Date Nov.2012	-0.72	0.34	-2.15	*
	Date_Feb.2013	-0.69	0.34	-2.03	*
	Date May.2013	-1.00	0.35	-2.82	**
	Date Aug.2013	-2.88	0.58	-4.94	***
	Date Nov.2013	-1.34	0.37	-3.59	***
	Date_Feb.2014	-1.01	0.35	-2.87	**
	Date May.2014	-1.69	0.41	-4.09	***
	Date Aug.2014	-0.99	0.35	-2.80	**
	Date Nov.2014	-1.36	0.38	-3.62	***

	Date_Feb.2015	-1.14	0.36	-3.15	**
	Date May.2015	-0.65	0.34	-1.93	.
	Date Aug.2015	-1.44	0.39	-3.73	***
	Date Nov.2015	-2.03	0.45	-4.57	***
	Date_Feb.2016	-0.81	0.35	-2.34	*
	Date May.2016	-1.70	0.41	-4.19	***
	Date Aug.2016	-1.62	0.40	-4.07	***
	Date Nov.2016	-1.74	0.41	-4.27	***
	Date Feb.2017	-2.49	0.51	-4.92	***
	Date May.2017	-2.49	0.51	-4.92	***
Pelican Banks North	(Intercept)	0.392	0.20	1.97	*
	Year_2012	-0.97	0.26	-3.71	***
	Year_2013	-0.71	0.25	-2.83	**
	Year_2014	-0.35	0.24	-1.44	
	Year_2015	-0.77	0.25	-3.05	**
	Year_2016	-1.64	0.30	-5.43	***
	Year_2017	-2.64	0.56	-4.73	***

C) Comparison of negative binomial models for the effect of season and year on total seed density, with the random effect of distance nested within transect. The best fit model selected for each site is in **bold**.

Site	Candidate models	df	AIC
Wiggins Island	**M4 = seeds ~ season + year + (1|transect:distance)**	**7**	**361.20**
	M5 = seeds ~ season*year + (1|transect:distance)	10	361.80
	M3 = seeds ~ year + (1|transect:distance)	6	364.80
	M2 = seeds ~ season + (1|transect:distance)	4	379.09
	M1 = seeds ~ 1 + (1|transect:distance)	3	381.40
Rodds Bay	**M5 = seeds ~ season*year + (1|transect:distance)**	**14**	**634.11**
	M3 = seeds ~ year + (1|transect:distance)	8	640.47
	M4 = seeds ~ season + year + (1|transect:distance)	9	642.47
	M1 = seeds ~ 1 + (1|transect:distance)	3	657.03
	M2 = seeds ~ season + (1|transect:distance)	4	658.93
Pelican Banks North	**M3 = seeds ~ year + (1|transect:distance)**	**8**	**466.54**
	M4 = seeds ~ season + year + (1|transect:distance)	9	468.48
	M1 = seeds ~ 1 + (1|transect:distance)	3	481.63
	M2 = season + (1|transect:distance)	4	483.22
	M5 = seeds ~ season*year + (1|transect:distance)	14	634.11

(D) Coefficients of best fit models for the effect of season and year on total seed density at Wiggins Island, Rodds Bay, and Pelican Banks North. Significance codes for p-values: "***" = 0.001; "**" = 0.01; "*" = 0.05; "." = 0.1; " " = 1.

Site		Estimate	SE	z-value	p-value
Wiggins Island	(Intercept)	-0.50	0.31	-1.62	
	Season_Senescent	0.61	0.25	2.39	*
	Year_2015	0.25	0.33	0.75	
	Year_2016	0.43	0.32	1.35	
	Year_2017	-1.69	0.50	-3.39	***
Rodds Bay	(Intercept)	-0.51	0.37	-1.37	
	Season_Senescent	1.21	0.39	3.09	**
	Year_2013	0.96	0.40	2.38	*
	Year_2014	0.63	0.41	1.52	
	Year_2015	0.49	0.42	1.16	
	Year_2016	0.80	0.41	1.96	.
	Year_2017	-0.86	0.55	-1.57	
	Season_Senescent x Year_2013	-1.53	0.53	-2.88	**
	Season_Senescent x Year_2014	-1.91	0.58	-3.29	***
	Season_Senescent x Year_2015	-0.72	0.53	-1.35	
	Season_Senescent x Year_2016	-2.07	0.57	-3.62	***
	Season_Senescent x Year_2017	-1.19	0.74	-1.61	
Pelican Banks North	(Intercept)	-0.36	0.32	-1.11	
	Year_2013	-0.11	0.43	-0.25	
	Year_2014	0.67	0.41	1.66	
	Year_2015	0.17	0.42	0.41	
	Year_2016	-0.87	0.49	-1.78	
	Year_2017	-1.87	0.63	-2.97	**

E) Comparison of poisson (Rodds Bay and Wiggins Island) and negative binomial (Pelican Banks) models for the effect of depth, month and year, and date as a proxy for month*year interaction, on total seed density. Random effect is distance nested within transect. The best fit model selected for each site is in **bold**.

Site	Candidate models	df	AIC
Wiggins Island	M8 = seeds ~ month + year + depth + (1|transect:distance)	10	523.31
	M4 = seeds ~ depth + (1|transect:distance)	**4**	**525.29**
	M7 = seeds ~ year + depth + (1|transect:distance)	7	525.64
	M6 = seeds ~ month + depth + (1|transect:distance)	7	525.75
	M9 = seeds ~ date + depth + (1|transect:distance)	15	532.21
	M5 = seeds ~ month + year + (1|transect:distance)	8	649.00
	M1 = seeds ~ 1 + (1|transect:distance)	2	650.95
	M2 = seeds ~ year + (1|transect:distance)	5	651.33
	M3 = seeds ~ month + (1|transect:distance)	5	651.44
Rodds Bay	**M4 = seeds ~ depth + (1|transect:distance)**	**4**	**796.52**
	M6 = seeds ~ month + depth + (1|transect:distance)	7	797.58
	M9 = seeds ~ date + depth + (1|transect:distance)	18	801.40
	M7 = seeds ~ year + depth + (1|transect:distance)	8	802.72
	M8 = seeds ~ month + year + depth + (1|transect:distance)	11	803.20
	M1 = seeds ~ 1 + (1|transect:distance)	2	922.91
	M3 = seeds ~ month + (1|transect:distance)	5	923.96
	M2 = seeds ~ year + (1|transect:distance)	6	929.10
	M5 = seeds ~ month + year + (1|transect:distance)	9	929.58
Pelican Banks North	**M4 = seeds ~ depth + (1|transect:distance)**	**5**	**607.78**
	M7 = seeds ~ month + depth + (1|transect:distance)	8	609.34
	M8 = seeds ~ month + year + depth + (1|transect:distance)	12	609.53
	M6 = seeds ~ year + depth + (1|transect:distance)	9	609.65
	M9 = seeds ~ date + depth + (1|transect:distance)	19	616.10

M1 = seeds ~ 1 + (1|transect:distance)	3	660.44
M3 = seeds ~ month + (1|transect:distance)	6	662.74
M2 = seeds ~ year + (1|transect:distance)	7	663.90
M5 = seeds ~ month + year + (1|transect:distance)	10	665.20

(F) Coefficients (fixed effects) of best fit models for the effect of depth, month and year, and date as a proxy for month*year interaction, on total seed density at Wiggins Island, Rodds Bay, and Pelican Banks North. Significance codes for p-values: "***" = 0.001; "**" = 0.01; "*" = 0.05; "." = 0.1; " " = 1.

Site		Estimate	SE	z-value	p-value
Wiggins Island	(Intercept)	-2.00	0.29	-6.94	***
	Depth_20-50mm	1.23	0.33	3.74	***
	Depth_50-100mm	2.40	0.30	7.98	***
Rodds Bay	(Intercept)	**-1.53**	**0.19**	**-7.92**	***
	Depth_20-50mm	0.84	0.23	3.72	***
	Depth_50-100mm	1.84	0.20	9.04	***
Pelican Banks North	(Intercept)	-1.80	0.26	-6.94	***
	Depth_20-50mm	1.49	0.29	5.07	***
	Depth_50-100mm	1.91	0.28	6.69	***

(G) ZOIB (mu) regression coefficients of best fit models for the effect of season and year on the proportion of viable seeds at Wiggins Island and Rodds Bay. Significance codes for p-values: "***" = 0.001; "**" = 0.01; "*" = 0.05; "." = 0.1; " " = 1.

Site		Estimate	SE	t-value	p-value
Wiggins Island	(Intercept)	$2.38x10^{-16}$	$2.08x10^{-1}$	0.00	
	Season_Senescent	-1.03	$2.67x10^{-1}$	-3.87	***
	Year_2016	-1.56	$3.43x10^{-1}$	-4.54	***
	Year_2017	-2.48	$2.81x10^{8}$	0.00	
Rodds Bay	(Intercept)	**1.56**$x10^{-16}$	**2.31**$x10^{-1}$	**0.00**	
	Season_Senescent	-1.11	$3.72x10^{-1}$	-2.97	**
	Year_2016	-1.14	$3.74x10^{-1}$	-3.04	**
	Year_2017	$4.38x10^{-1}$	$6.63x10^{7}$	0.00	
	Season_Senescent x Year_2016	1.69	$5.66x10^{-1}$	2.98	
	Season_Senescent x Year_2017	1.39	$1.06x10^{8}$	0.00	