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Moisture content, temperature, and relative humidity influence seed storage and subsequent survival and germination of *Vallisneria americana* seeds

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ABSTRACT

Loss of aquatic plant species has occurred in coastal and freshwater habitats throughout the world. In particular, population and habit loss of the submerged aquatic species, *Vallisneria americana* Michx., have made it a potential restoration candidate. For successful restoration, reliable seed sources are vital for producing plants or for direct seeding. Seed survival and their ability to retain viability through storage are key components for successful reproduction. Seed storage and subsequent germination of *V. americana* were studied over a 6-month period to determine the conditions that retain seed vigor. Seeds were stored at three humidity levels 11%, 50%, 95% using saturated salts and ambient humidity for 1, 2, 4, and 6 months at either cold or ambient temperatures. After storage intervals, seed moisture content, embryo viability, and germination were determined. Timing of germination and seed survival were analyzed using semi- and non-parametric analyses. Storing seeds at lower RH levels for longer periods reduced seed survival and resulted in later germination. Germination and seed survival increased in seeds stored at higher relative humidity and led to earlier germination. Seeds of *V. americana* from the northern Gulf of Mexico were shown to be desiccation tolerant and have orthodox seed characteristics.

1. Introduction

Vallisneria americana Michx. is a perennial, submerged aquatic macrophyte, found in coastal and freshwater aquatic ecosystems throughout North America, and is a focal species for restoration in the Chesapeake Bay and Gulf of Mexico regions (Lloyd et al., 2012). Due to natural and anthropogenic events (Short and Wyllie-Echeverria, 1996; Short and Neckles, 1999; Hay et al., 2000), maintaining germplasm (e.g. seeds) is necessary when natural seed production is low or unavailable (Ailstock and Shafer, 2006). In particular, determining proper seed storage techniques is vital to retain viability through desiccation and subsequent storage (Young and Young, 1986; Tweddle et al., 2003).

Seed storage can be challenging given the inherent nature of seeds. Orthodox seeds can survive desiccation to 3–7% moisture content and temperatures as low as −20 °C for an indefinite period (Hay et al., 2000). Recalcitrant or unorthodox seeds cannot germinate when desiccated below 20–40% moisture content (Hay et al., 2000). Still other seeds have intermediate storage characteristics and can be dried to 12–14% moisture content, but cannot be stored cold upon desiccation (Hay et al., 2000; Bonner, 2008). While unorthodox storage characteristics in aquatic seeds is common, orthodoxy may be more frequent than previously thought (Hay et al., 2000).

Seed storage of *V. americana* has been previously studied, but debate on the subject has not been concluded. Muenscher (1936) found that mature seeds could not be desiccated in storage without damaging the embryo, but seeds remained viable when intact capsules were stored at 4 °C for up to 3 years (Ferasol et al., 1995; Campbell, 2005; Moore and Jarvis, 2007). In a related species, *V. australis* S.W.L Jacobs & D.H. Les, from central Victoria, Australia, drying seeds up to 8 months more than doubled the final germination percentage but also delayed germination compared to wet-stored seeds (Salter et al., 2010).

Here we describe a technique to desiccate and store *V. americana* seeds under different relative humidity (RH) levels and temperatures. Although seed storage of *V. americana* is unclassified, this species may have non-orthodox seed storage characteristics (Hay et al., 2000). Seeds can remain viable when capsules are stored

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in water, but doing so can lead to contamination and precocious germination, and timing collection of mature capsules can be challenging. Our objectives were to determine: (1) whether *V. americana* seeds can survive desiccation, (2) conditions that allow *V. americana* seeds to be stored for up to 6 months, and (3) seed storage physiology of *V. americana* seeds.

2. Materials and methods

2.1. Seed source and collection

During November 2012, approximately 75 mature seed capsules were collected from tank-grown plants located at the Gulf Coast Research Laboratory, Ocean Springs, MS. The tanks were under 50% shade cloth, but exposed to natural temperature and photoperiod conditions. Upon collection seeds were immediately removed from capsules, and placed in water at ambient temperature to allow the mucilage to naturally degrade (5–7 days). Prior to experimentation, all seeds were pooled from the collected capsules.

2.2. Experimental design

The seed storage system used was similar to Carpenter et al. (1995). Seeds were placed on a 70 mm diameter filter paper supported by a 60 mm × 15 mm piece of PVC pipe in 100 mm × 25 mm Petri dishes (D943; *Phyto*Technology Laboratories®, LLC, Shawnee Mission, KS). Two Petri dishes (one for germination and viability testing and one for moisture content determination) containing 250 seeds each were prepared per storage treatment (Fig. 1). To reduce storage variability, seeds allocated for germination were pretreated in one dish as per standard methods (Ellis et al., 1982, 1989). Storage treatments were a combination of two temperatures (3.0 °C ± 1.5 or ambient temperature (23.2 °C ± 3.0)), four RH conditions, and four storage storage times (1, 2, 4, or 6 months). The four RH conditions were achieved by allocating 50 ml of a saturated salt solution (95% RH-KNO_3, 50% RH-$Mg(NO_3)_2$, 11% RH-LiCl, ambient of 50.2% ± 9.6 RH) to each dish (Wexler and Hasegawa, 1954; Greenspan, 1977). Petri dishes were sealed with Parafilm® (Pechiney Plastics Packaging Company, Chicago, IL) and stored in continuous darkness.

After designated storage times, seeds from the first Petri dish were dried at 130 °C for 1 h and cooled for 45 min at 31.3% ± 7.4 RH (International Seed Testing Association, 1985). Seed moisture content of two replications (125 seeds each) was determined using the following equation:

$$(M2 - M3) \times \frac{100}{M2 - M1}$$

where M1 is the weight in grams of the container and its cover; M2 is the weight in grams of the container, its cover, and seeds before drying; and M3 is the weight in grams of the container, its cover, and seeds after drying.

From the second Petri dish, seeds were randomly selected for viability and germination testing. Embryo viability of 100 seeds was tested after designated storage times using a 1% 2,3,5-triphenyl tetrazolium chloride solution (Lakon, 1949). To facilitate embryo staining, seed coats were nicked at the distal end of the seed with a razor blade. Seeds were incubated at 30 °C in the dark for 48 h before examination. Embryos were considered viable if any degree of red staining was observed.

Germination tests were conducted by monitoring three replications of 50 seeds for each storage treatment combination (temperature × RH × storage time). Seeds were agitated alternately twice in 95% ethanol and distilled water for 3 min to reduce potential contamination (Seeliger et al., 1984). Seeds were then placed in 100 mm × 15 mm petri dishes with 20–25 ml water, and incubated in VWR Signature™ Diurnal Growth Chambers (model 2015, VWR International, Radnor, PA, USA) with a 16 h photoperiod (GE Ecolux 4100k cool-white fluorescent tubes; F32T8SP41; General Electric, Fairfield, CT, USA) at 30.1 °C ± 2.2. Water was replenished as needed.

2.3. Data collection and analysis

Temperature and RH levels were monitored throughout the experiment using HOBO HO8-003-02 data loggers (Onset Computer Corporation, Bourne, MA). Germination was monitored every 2–3 days for 30 days. Due to a large percentage of censured data (no germination), semi-parametric and non-parametric time-to-event analyses were conducted (McNair et al., 2012; Perez and Kettner, 2013). After the experimental period, germinated seeds were assigned an event code of '1' and non-germinated seeds were assigned '0'.

The non-parametric Kaplan–Meier estimator of survivor function (Kaplan and Meier, 1958) was used to determine how likely seeds survived during the course of the experiment. Main effects (RH, month, temperature) along with temperature/RH interactions within months were tested. Data were log-ranked and the Holm–Sidak post hoc multiple comparison test was used to compare final survival data. Kaplan–Meier statistics were generated in both R v. 2.12 and SigmaPlot v. 12, while Pearson's correlation test was performed in SigmaPlot v. 12.

The semi-parametric Cox proportional hazards model (Cox, 1972) was used to determine how likely it is that an ungerminated seed will germinate within the experimental time frame (30 days). This is referred to as the hazard function or rate and replaces traditional measures such as germination rate or mean germination time. A forward model building approach was used in R v. 2.12 (http://www.r-project.org/) to determine the combination of the factors (including interaction effects) that gave the best overall Cox model using the fewest variables. A three factor model using month, relative humidity, and temperature was determined to be the best fit model based on the Likelihood Ratio. Using the Cox regression proportional hazards model function in SigmaPlot v. 12 (Systat Software, Inc, Chicago, IL), a Chi-square test with a Holm–Sidak correction was used to compare main effects (month, temperature, treatment) to the reference group. Once the hazard ratio coefficients were calculated, the values were used to construct a look up table in Excel 2010 (Microsoft Inc, Redmond, WA, USA)

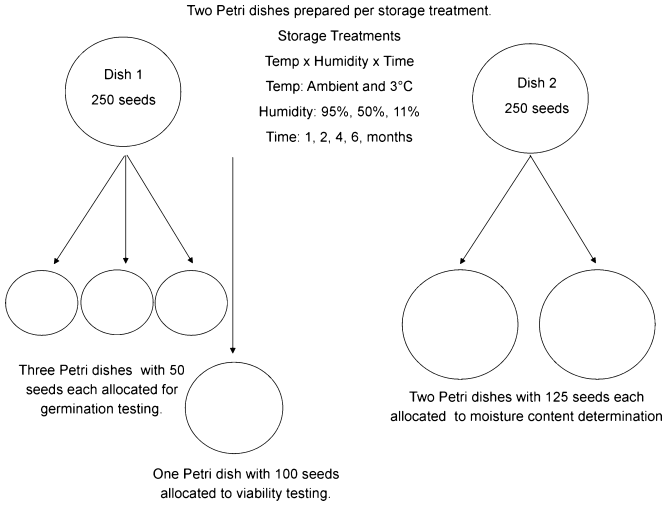

Fig. 1. Outline of experimental procedures.

Table 1
Germination, viability, and moisture content of *V. americana* seeds after designated storage times and conditions.

Treatment	Final germination[a]	Seed viability[b]	Seed moisture content[c]
95% humidity			
Cold temp			
Month 1	4.76 ± 1.15	68.8	23.2 ± 1.3
Month 2	8.67 ± 3.06	70.0	27.1 ± 0.5
Month 4	10.67 ± 1.15	66.6	28.3 ± 0.5
Month 6	20.67 ± 5.03	58.0	32.1 ± 0.3
Ambient temp			
Month 1	10.67 ± 5.03	80.0	17.1 ± 0.3
Month 2	4.67 ± 1.15	80.0	26.0 ± 7.4
Month 4	6.67 ± 1.15	88.0	26.4 ± 0.5
Month 6	10.0 ± 6.92	76.0	25.8 ± 0.6
50% humidity			
Cold temp			
Month 1	12.67 ± 4.16	100.0	15.2 ± 0.5
Month 2	8.67 ± 3.06	70.0	16.8 ± 0.7
Month 4	7.33 ± 1.15	66.0	13.2 ± 0.4
Month 6	8.00 ± 2.00	56.0	13.2 ± 0.3
Ambient temp			
Month 1	8.76 ± 4.62	100.0	14.9 ± 0.7
Month 2	7.33 ± 1.15	80.0	14.8 ± 0.3
Month 4	3.33 ± 2.31	80.0	14.4 ± 1.0
Month 6	10.67 ± 1.15	70.0	13.4 ± 0.2
Ambient humidity			
Cold temp			
Month 1	20.67 ± 4.16	83.3	8.8 ± 0.8
Month 2	12.00 ± 6.93	64.0	7.1 ± 0.9
Month 4	4.00 ± 2.00	64.0	8.7 ± 0.4
Month 6	10.67 ± 7.02	60.0	5.1 ± 0.4
Ambient temp			
Month 1	8.00 ± 0	90.0	10.5 ± 0.7
Month 2	10.67 ± 2.31	90.0	9.3 ± 0.3
Month 4	10.67 ± 2.31	76.0	11.0 ± 0.8
Month 6	12.67 ± 3.06	50.0	10.8 ± 0.1
11% humidity			
Cold temp			
Month 1	16.7 ± 11.7	65.0	8.6 ± 0.8
Month 2	8.67 ± 1.15	60.0	4.7 ± 0.9
Month 4	5.33 ± 2.33	50.0	9.3 ± 0.6
Month 6	3.33 ± 2.31	34.0	7.0 ± 0.3
Ambient temp			
Month 1	6.67 ± 2.31	91.8	10.5 ± 0.5
Month 2	4.00 ± 0	88.0	5.8 ± 0.6
Month 4	5.33 ± 3.06	86.0	10.1 ± 1.02
Month 6	6.00 ± 2.00	76.0	3.8 ± 1.2

[a] Final germination percent ± standard error is the average of 150 seeds divided equally among three petri plates.
[b] Seed viability is the average percent viability of a 50 seed aliquot.
[c] Percent moisture content is the average of two replicates of 125 seeds each.

to determine current and future germination times. Seeds stored at 95% RH for 1 month at ambient temperature were used as the reference group.

3. Results

3.1. Germination, viability, and moisture content

Final germination ranged from 3.33% to 20.67% (Table 1). At 3 °C, germination decreased with time in storage at 50% RH, ambient RH, and 11% RH. When stored at ambient temperature, germination increased slightly with time in storage at 50% and ambient RH and was similar from 1 to 6 months in storage at 95% and 11% RH. Freshly collected and mature seeds were on average 86.5% viable. Seed viability decreased with time in storage with all treatments (Table 1).

Table 2
Results of Kaplan–Meier log rank survivor estimate test results. Significant results ($\alpha < 0.05$) are in bold.

Test	Chi square	DF	*p*
Humidity treatment	13.63	3	**0.003**
11% vs ambient	12.29		**0.003**
95% vs 11%	5.55		0.09
50% vs ambient	5.49		0.07
95% vs 50%	1.45		0.54
11% vs 50%	1.37		0.43
95% vs ambient	1.20		0.27
Month	24.33	3	**<0.0001**
1 vs 2	14.39		**0.001**
1 vs 4	13.36		**0.001**
2 vs 6	10.51		**0.004**
4 vs 6	9.67		**0.006**
1 vs 6	0.24		0.86
2 vs 4	0.03		0.90
Temperature	7.76	1	**0.01**
Month 1			
Ambient temperature	1.68	3	0.64
3 °C	17.08	3	**<0.0001**
95% RH vs Ambient RH	16.62		**0.0003**
95% RH vs 11% RH	10.78		**0.005**
Month 2			
Ambient temperature	6.66	3	0.08
3 °C	1.01	3	0.79
Month 4			
Ambient temperature	7.34	3	0.06
3 °C	6.40	3	0.09
Month 6			
Ambient temperature	4.08	3	0.25
3 °C	27.05	3	**<0.0001**
95% RH vs 11% RH	21.77		**<0.0001**
95% RH vs 50% RH	10.10		**0.007**
95% RH vs ambient RH	6.18		**0.04**
11% RH vs ambient RH	6.29		**0.04**

The average moisture content of freshly collected, mature seeds of *V. americana* was 60.9 ± 3.9%. After storage times average seed moisture content ranged from 3.8 to 32.1% (Table 1). Average seed moisture content was highest in the 95% RH treatment (25.8 ± 5.0%) and lowest in the 11% RH treatment (7.5 ± 2.6%). Moisture content increased when seeds were stored at 95% RH regardless of temperature, and remained the same when stored at both ambient temperature and RH. Moisture content decreased with time in storage with all other treatments (Table 1).

3.2. Survivor estimates

Humidity treatments, month in storage, and temperature, all had significant effects on seed survival (Table 2). Seed survival curves for humidity treatments decreased continuously (Fig. 2A), with survival of seeds stored at 95% RH diverging by day 2 from the other treatments. Survival curves for ambient, 50%, and 11% RH diverged between day 15 and 20. By day 30, survival curves were similar between all RH treatments except 11% and ambient RH treatments (Table 2). Seed survival curves for months in storage remained grouped until day 20 when months 1 and 6 decreased rapidly compared to months 2 and 4 (Fig. 2B). Curves for months 1 and 6 as well as 2 and 4, respectively, were not significantly different from each other (Table 2). Seed survival curves for temperature diverged at day 20 (Fig. 2C) and were significantly different.

Further analysis of month and temperature interactions revealed that ambient temperature did not influence seed survival regardless of month, and storage at 3 °C after 2 and 4 months did not influence seed survival either (Table 2). Survival at ambient temperature followed similar curves for the different RH treatments within each month (Figs. 3 and 4), whereas seeds in cold storage exhibited RH-dependent survival. At 3 °C after 1 month storage, seed survival

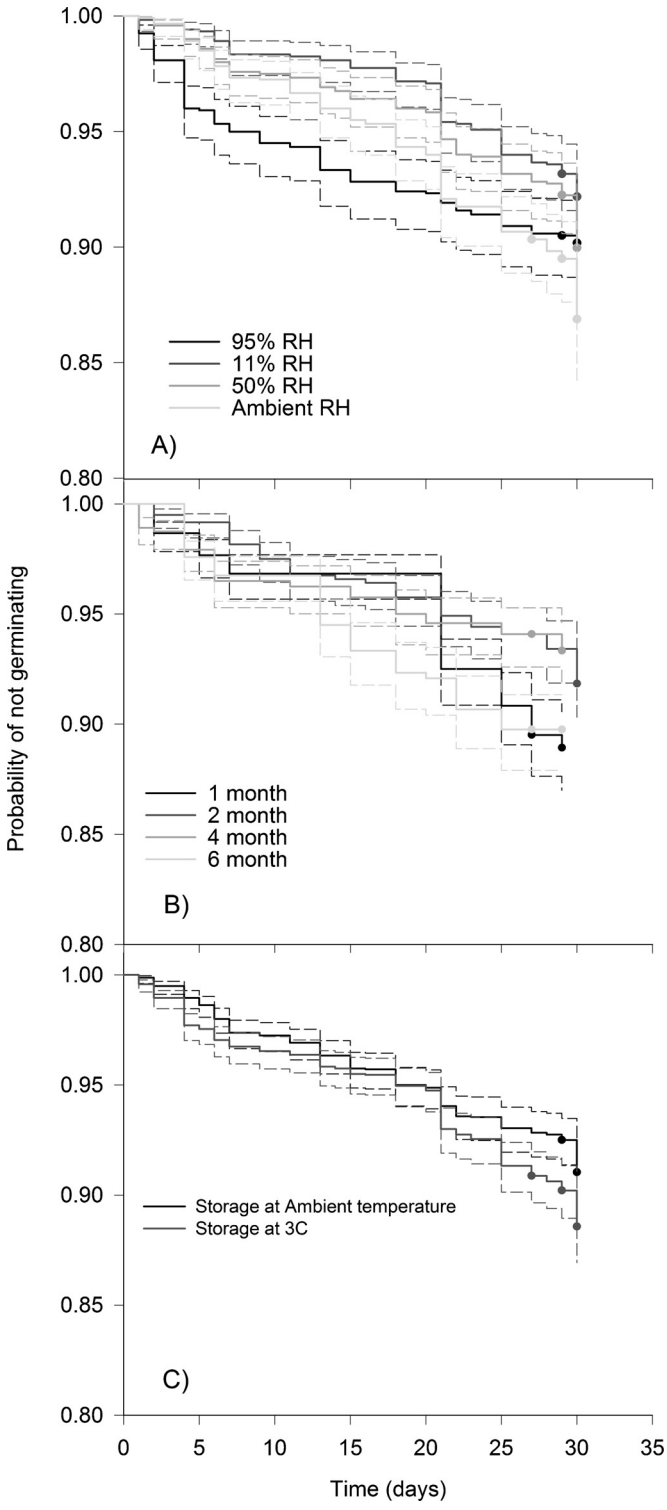

Fig. 2. Kaplan–Meier survivor main effects curves of *V. americana* seeds stored at (A) three humidity levels 11%, 50%, 95% using saturated salts and ambient humidity, (B) for 1, 2, 4, and 6 months, (C) at either cold (3 °C) or ambient (23 °C) temperatures.

differed between 95% and ambient (25%) RH, as well as 95% and 11% RH (Table 2). Major survival curve divergence occurred at day 20 with decreased survival at 11% and 25% RH compared to 50% and 95% RH (Fig. 3C). After 6 months storage at 3 °C, seed survival at 95% RH differed from all other RH treatments (Table 2). The survival curve for the 95% RH seeds at 3 °C diverged within 5 days from the other three RH treatment curves and decreased rapidly (Fig. 4D). In addition, the survival curves for 11% and 25% RH diverged around day 12 (Fig. 4D) and differed significantly at day 30 (Table 2).

Table 3
Summary table of the final Cox model[a] for the *V. americana* seed storage data, as produced by R function coxph()[b]. SE denotes the standard error. Multiple comparisons of the main effects were compared to reference group. Significant results ($\alpha < 0.05$) are in bold.

Covariate, *xi*	Coefficient, βi	exp(βi)[f]	SE of βi	*z*	*p*
Month[c]	−0.227	0.797	0.062	−3.674	**0.000**
Month 1	0.509	1.664	0.141		**0.001**
Month 2	−0.020	0.980	0.159		0.899
Month 6	0.446	1.562	0.144		**0.002**
Temp[d]	−0.018	0.983	0.006	−3.006	**0.003**
3 °C	−0.268	0.765	0.097		**0.006**
RH treatment[e]	−0.010	0.990	0.003	−2.933	**0.003**
50% RH	−0.337	0.714	0.143		**0.019**
11% RH	−0.166	1.150	0.127		0.273
Ambient RH	0.139	0.847	0.137		0.225
Month × RH	0.004	1.004	0.001	4.761	**<0.0001**

[a] Likelihood ratio test = 37.93 on 4 df, $p = 1.157e^{-07}$; Wald test = 38.22 on 4 df, $p = 1.008e^{-07}$.
[b] coxph(formula = Surv(day, status) ~ month + temp + treatment + treatment:month, data = all months). Month, temperature, treatment, and month × trt all had significant effects on germination.
[c] Month 4 served as reference group.
[d] Ambient temperature served as reference group.
[e] 95% humidity served as reference group.
[f] Hazard ratios exp(βi) less than 1 mean seeds germinate later than the reference group, whereas hazard ratios greater than 1 mean seeds germinate earlier.

3.3. Germination models and hazard rates

Time in storage, temperature, RH treatment, and month × RH interaction all had a significant influence on germination probability (Table 3). The overall Cox model suggested that seeds stored at lower temperatures were more likely to germinate later than those stored at warmer temperatures, and that higher RH (wetter conditions) promoted the likelihood of earlier germination than seeds stored dry. The likelihood of germination earlier than 30 days of seeds stored for 1 month (1.66 as likely) and 6 months (1.56) was greater than seeds stored for either 2 or 4 months (Table 3). Seeds stored at 3 °C were more likely to germinate after 30 days compared to seeds stored at ambient temperature (Table 3). Compared to moist storage at 95% RH, seeds stored at 50% and ambient RH were more likely to germinate after the 30 day experimental time frame, whereas seeds stored drier at 11% RH were more likely to germinate earlier. However, only storage at 50% RH was significantly different (Table 3).

Further analyzing the Cox model by each month separately revealed no significant influence of temperature, RH, or their interaction on seed germination after 2 and 4 months storage (Table 4). However, temperature, RH, and their interaction influenced germination after 1 month storage, while only temperature and the temperature × RH interaction were highly significant after 6 months storage, although RH treatment was barely non-significant (Table 4).

Using the Cox model to predict seed germination likelihood after prolonged storage (Table 5) indicated that after 8, 10, and 12 months storage time, germination continued to follow the previously established patterns. At 11% and 50% RH storage, germination was predicted to happen later when seeds were stored at 3 °C but earlier when stored at higher temperatures compared to the reference group (95% RH at 1 month at ambient temperature). The model also indicated that if seeds were to be stored at 95% RH for 8 months or longer, regardless of temperature, that germination would be earlier compared to storing seeds at 95% RH and ambient temperature for just 1 month. The data for 8, 10 and 12 months presented in this table should be used with a degree of caution as it

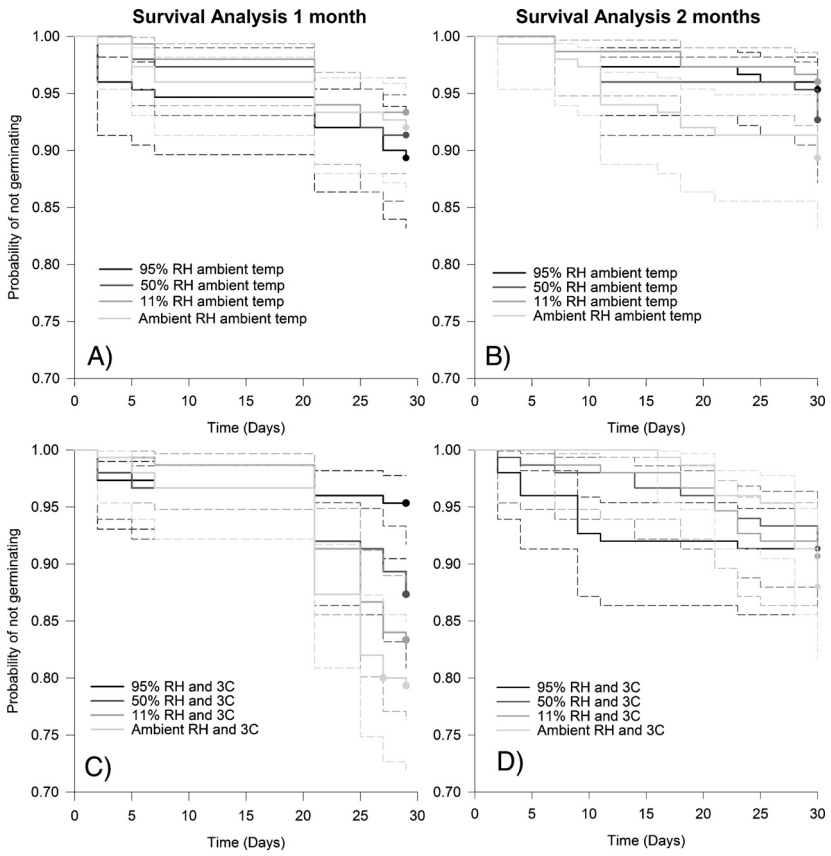

Fig. 3. Kaplan–Meier survivor interaction effect curves of *V. americana* seeds stored at ambient (23 °C) temperature for 1 month (A) and 2 months (B) or at cold (3 °C) temperature for 1 month (C) and 2 months (D) over three humidity levels 11%, 50%, 95% using saturated salts and ambient humidity.

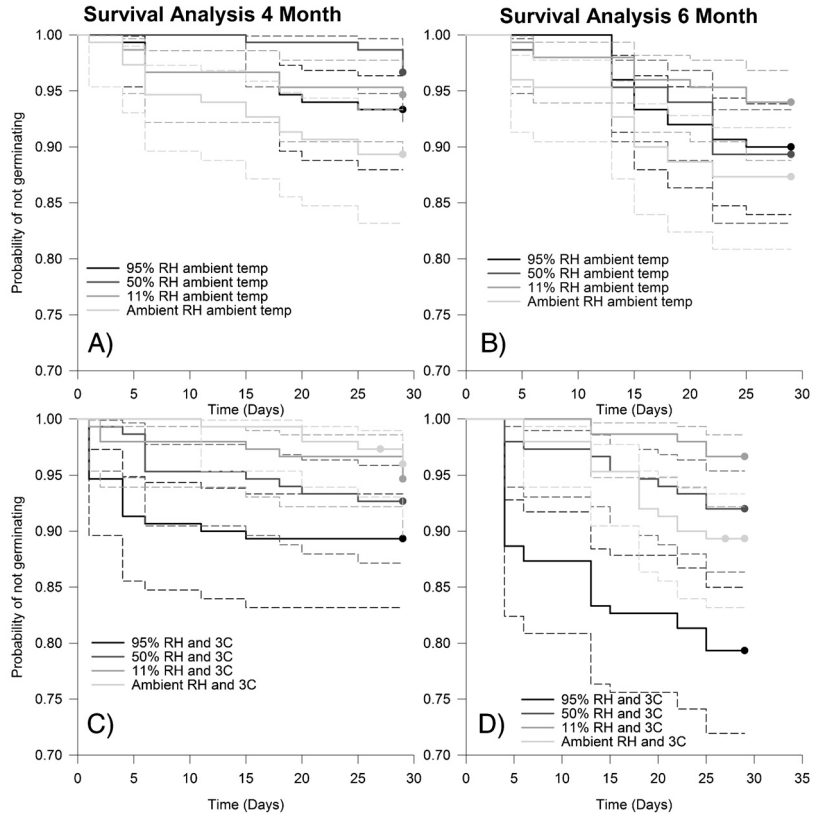

Fig. 4. Kaplan–Meier survivor interaction effect curves of *V. americana* seeds stored at ambient (23 °C) temperature for 4 months (A) and 6 months (B) or at cold (3 °C) temperature for 4 months (C) and 6 months (D) over three humidity levels 11%, 50%, 95% using saturated salts and ambient humidity.

Table 4
Summary tables of monthly Cox models[a] for the *V. americana* seed storage data, as produced by R function coxph(). SE denotes the standard error. Significant results ($\alpha < 0.05$) are in bold.

Covariate, *xi*	Coefficient, βi	exp(βi)	SE of βi	*z*	*p*
Month = 1					
Temp	−0.019	0.981	0.006	−3.299	**0.001**
RH treatment	−0.061	0.941	0.019	−3.155	**0.002**
Temp × RH	0.001	1.001	0.000	3.048	**0.002**
Month = 2					
Temp	−0.001	0.999	0.006	−0.161	0.872
RH treatment	−0.031	0.969	0.023	−1.325	0.185
Temp × RH	0.001	1.001	0.000	0.234	0.815
Month = 4					
Temp	0.010	1.010	0.006	1.614	0.107
RH treatment	−0.006	0.994	0.027	−0.232	0.817
Temp × RH	−0.000	0.999	0.000	−0.706	0.480
Month = 6					
Temp	0.028	1.028	0.006	4.412	**<0.0001**
RH treatment	0.049	1.050	0.025	1.930	0.054
Temp × RH	−0.001	0.999	0.000	−2.663	**0.008**

[a] Month = 1: likelihood ratio test = 13.83 on 3 df, $p = 0.003$; Wald test = 14.03 on 3 df, $p = 0.003$; month = 2: likelihood ratio test = 4.46 on 3 df, $p = 0.216$; Wald test = 4.31 on 3 df, $p = 0.23$; month = 4: likelihood ratio test = 6.47 on 3 df, $p = 0.091$; Wald test = 6.85 on 3 df, $p = 0.077$; month = 6: likelihood ratio test = 28.84 on 3 df, $p = 2.415e^{-06}$; Wald test = 28.2 on 3 df, $p = 3.292e^{-06}$.

extrapolates the Cox regression beyond the initial dataset and seed viability may decrease below that established by the experiments conducted up to 6 months storage.

4. Discussion

Vallisneria americana seed tolerated varying degrees of desiccation depending on storage time, RH, and temperature, and were found not to be recalcitrant and did not lose the ability to germinate after cold storage as intermediate seeds do (Ellis et al., 1990). *V. americana* seeds from the northern Gulf of Mexico were found to have characteristics of orthodox seeds tolerating desiccation to 4% moisture content. However, as storage time increased, seeds required moisture content above 25% to achieve higher germination percentages. While many aquatic species have been reported to have recalcitrant seeds, they have varying degrees of tolerance to drying, which is often a requirement for survival in wetlands (Aldridge and Probert, 1992; Hay et al., 2000).

Temperature had varying effects on seed storage characteristics. While embryo viability was higher at ambient temperature, germination and survival was higher when seeds were stored at 3 °C. Cold storage has been shown to improve germination of numerous aquatic species including *Ruppia maritima* L. and *Potamogeton perfoliatus* L. (Ailstock and Shafer, 2006), *Zostera capricorni* Asherson (Conacher et al., 1994), and numerous other species (see Muenscher, 1936). *Zostera capricorni* seeds were 58% viable after 50 days cold storage, but no seeds were viable after 50 days when stored between 22 and 24 °C. Improved germination with cold stored *V. americana* seeds was not surprising as a 5 °C drop in temperature can double the lifespan of seeds (Harrington, 1972).

During storage, protein and nucleic acid biosynthesis declines (Bray and Chow, 1976; Cruz-Garcia et al., 1995), and oxidative reactions, such as development of peroxidases, contribute to decreased seed vigor (Bernal-Lugo et al., 2000). At higher storage temperatures increased concentrations of peroxidases damage seed structure leading to decreased seed vigor, and a lack of antioxidants fail to protect seeds from excessive damage (Kandil et al., 2013; Liaotrakoon et al., 2013). Still, this does not explain the higher seed viability observed at ambient temperature in *V. americana* seeds, as viability and vigor generally decline with increasing temperature (Justice and Bass, 1978). During cold storage, certain metabolic processes of *V. americana* seeds could have been slowed or inhibited so that tetrazolium did not stain embryos effectively. The possibility that *V. americana* seeds from the northern Gulf of Mexico do lose viability at colder temperatures also exists as low winter temperatures are rare in this region (Crane et al., 2003).

Temperature and RH influenced seed moisture content and ultimately seed survival. As seed moisture content approaches a range between 5 and 14%, the lifespan of a seed can double (Harrington, 1972). In orthodox seeds, lipid breakdown is enhanced at higher temperatures as moisture content declines (McDonald, 2004). Free radicals deteriorate lipids in seeds below 6% and above 14% moisture content, but between 6 and 14% moisture content water is sufficient to buffer against the negative effects of free radicals (McDonald, 2004). In our study, *V. americana* seeds had higher germination percentages and rates with higher moisture content. In fact, seed survival in the 95% RH treatment increased over the course of the experiment likely due to the higher moisture content (from 17 to 32%). However, seed survival was higher in the short term at 3 °C and 11% RH suggesting that induced dormancy may occur in seeds stored at lower temperature and RH.

Desiccation tolerance of seeds is a common feature of aquatic plants found in seasonal or temporary wetlands (Leck and Brock, 2000). While *V. americana* is a widespread species in North America, many populations are found in lakes and estuaries that have a year round supply of water (pers. obs.). The populations in the shallow coastal waters along the northern Gulf of Mexico are subject to frequent periods of prolonged exposure (drawdown) during winter low tides (pers. obs.). Desiccation tolerant seeds may persist during this drawdown, and may result in population re-establishment from seed when mature plants would otherwise die (Salter et al., 2010). However, seed viability in many aquatic species decreases over time when exposed to air (Leck and Brock, 2000; Brock et al., 2003).

Numerous submerged aquatic species have seed coats that inhibit germination and require scarification before germination (Orth et al., 2000) including *Najas marina* (Agami and Waisel, 1986, 1988), *Zostera marina* (Moore et al., 1993), and *V. americana* (Kauth

Table 5
Predicted germination timing of *V. americana* seeds based on Cox proportional hazard ratio model. Data were compared to storage at 95% RH at ambient (23 °C) temperature after 1 month storage.

Month	11% humidity			50% humidity			95% humidity		
	3C	25C	40C	3C	25C	40C	3C	25C	40C
1	Later	Later	Later	Later	Later	Later	Later	Same	Earlier
2	Later	Later	Later	Later	Later	Earlier	Later	Earlier	Earlier
4	Later	Later	Earlier	Later	Earlier	Earlier	Later	Earlier	Earlier
6	Later	Earlier	Earlier	Later	Earlier	Earlier	Later	Earlier	Earlier
8	Later	Earlier	Earlier	Later	Earlier	Earlier	Earlier	Earlier	Earlier
10	Later	Earlier	Earlier	Later	Earlier	Earlier	Earlier	Earlier	Earlier
12	Later	Earlier	Earlier	Later	Earlier	Earlier	Earlier	Earlier	Earlier

and Biber, 2014). The restrictive nature of the seed coat of *V. americana* seeds likely contributed to the variability in germination over all treatments. Scarifying the seed coat after storage may contribute to higher germination percentages as seen in non-stored matured seeds (Kauth and Biber, 2014).

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