

Overwinter Storage of Eelgrass (*Zostera marina*) Seeds from a Nova



Scotian Population for use in Coastal Habitat Restoration

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Background & Research Gap

Eelgrass (*Zostera marina*) is a habitat-forming foundation species that sequesters carbon, prevents coastal erosion, improves water quality, and fosters biodiversity (Unsworth et al. 2022).



Figure 1 Eelgrass under sea ice in New Brunswick (Nicholas Winkler Photography).



Figure 2 European green crabs are invasive in NS and destroy eelgrass beds.

- Accelerating worldwide decline of eelgrass meadows due to anthropogenic factors (Waycott et al. 2009).
- **Seed-based restoration:** lower impacts on donor meadows, increased genetic diversity in restored meadows, may be more cost-effective (Busch et al. 2008).
- NS eelgrass populations go into seed mid-late summer, but seeds don't germinate until the following spring after an overwinter period (Vercaemer et al. 2021).
- **NS seeds must be stored overwinter to be used in restoration efforts.**
- Eelgrass biology varies by population and region.

Overwinter seed storage has yet to be studied for Nova Scotian eelgrass populations.

Research Question

What storage conditions will best preserve viability for Nova Scotian eelgrass seeds?

Objective

Pilot experiment investigating ideal storage conditions for eelgrass seeds from NS populations.

Hypotheses

- Higher salinity and colder temperature will best preserve seed viability.
- Copper sulfate and aeration will enhance these effects.



Figure 3 Eelgrass spathe on a reproductive shoot bearing unripe seeds, Aug 2025.



Figure 4 Ripe eelgrass seed with hard seed coat, Nov 2025.



Figure 5 Prematurely germinating eelgrass seed with embryo & cotyledon emerging from seed coat.

Project Overview



Experimental Setup

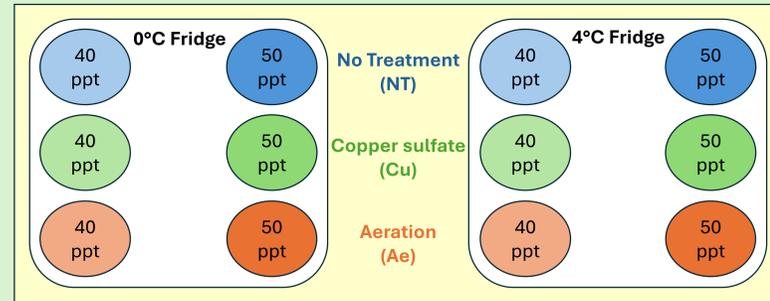


Figure 6 Experimental set up crossing 40 ppt & 50 ppt with 0°C & 4°C, replicated with No Treatment (NT), Copper Sulfate (Cu), and Aeration (Ae) for a total of 12 distinct storage treatments.



Figure 7 Division of ripe eelgrass seeds into ~180 seeds per jar. 1/8 tsp used to estimate 0 seeds.



Figure 8 Experiment set up with 12 distinct storage treatments, 3 replicates jars each. Half in a 0°C fridge (left) and half in a 4°C fridge (right).

Viability Test

- Remove 10 seeds per jar every 2 weeks to stain with triphenyl tetrazolium chloride (TTC).
- Red/pink tissue = viable.



Figure 9 Viable Seeds: red/pink staining of embryo. Embryos partially removed from seed coats for visibility.



Figure 10 Unviable Seeds: embryos not intact (left), or intact but unstained (right).

References

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Preliminary Results & Analysis

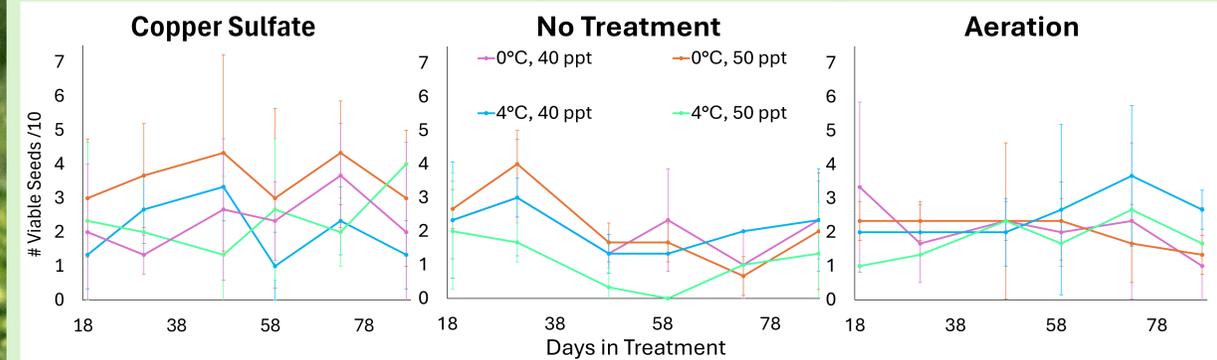


Figure 11 Mean (+ SD) number of viable eelgrass (*Z. marina*) seeds out of a subsample of 10 for every treatment biweekly throughout storage. 0°C-40 ppt, 0°C-50 ppt, 4°C-40 ppt, and 4°C-50 ppt were replicated across three different treatment variations for a total of 12 treatments.

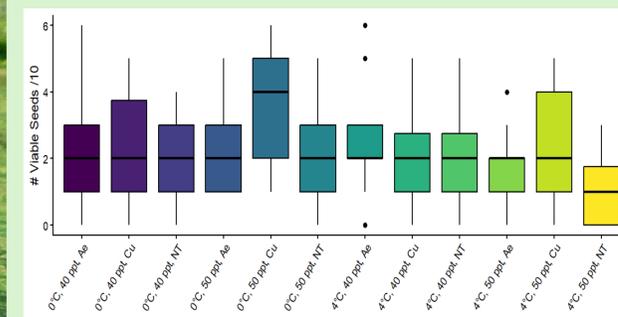


Figure 12 Summary statistics of all seed viability observations throughout storage for all 12 storage treatments. Ae = Aeration, Cu = Copper sulfate, NT = No Treatment.

Table 1 Results of a Generalized Least Squares ANOVA with separate residual variances for each Treatment level (NT, Cu, & Ae).

| Effect | F | p | Significance |
|-------------------------------|-------------|---------------|---------------------------------|
| Salinity | 0.61 | 0.4364 | No main effect |
| Temperature | 3.54 | 0.0613 | Marginal trend, not significant |
| Treatment | 4.54 | 0.0118 | Significant main effect |
| Salinity × Temperature | 5.13 | 0.0246 | Significant interaction |
| Salinity × Treatment | 3.80 | 0.0240 | Significant interaction |
| Temperature × Treatment | 1.57 | 0.2116 | Not significant |
| 3-way interaction | 0.10 | 0.9026 | Not significant |

Because residual variance differed by Treatment, a generalized least squares model with a Treatment-specific variance structure was applied. This model significantly improved fit (LRT $p = 0.0405$). Inference is therefore based on the GLS ANOVA (Table 1).

Discussion

- Prolonged decay period before starting storage experiments likely contributed to >90% infection of seeds and general low viability.
- Wide standard deviation and fluctuation of seed viability over time (Fig. 11) is likely due to small subsample (10 seeds per jar) and number of replicates (averaged out of 3 jars).
- TTC viability stain protocol (Figs. 9, 10) has yet to be standardized for eelgrass seeds.

Conclusions

- Treatment was the most significant factor in seed viability (Table 1). Copper sulfate (Cu) in particular presented wider and higher ranging variation in seed viability (Figs. 11, 12).
- Salinity x Temperature and Salinity x Treatment interactions were also significant factors in seed viability. **0°C 50 ppt Copper sulfate** presented the highest ranging viability overall (Figs. 11, 12).

Future Work

- Larger subsamples, more replicates per treatment.
- Germination tests alongside TTC staining for a better understanding of staining protocol and seed viability.
- Focus on high salinity, low temperature, copper sulfate, and aeration.

Acknowledgements

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