Standard Operating Procedures (3.4): Seed Bank Germination

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Standard Operating Procedures:
Seed Bank Germination

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement seed bank germination protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of seed bank survey protocols can be found in Appendix 3.4A.

Table 1. Appropriate habitat types to implement seed bank survey protocols.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Seed Bank	X (edge)	X (edge)	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for seed bank survey protocols.

	Evaluation Metric	Seed Bank	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Site selection and any GPS locations; print data sheets
	Equipment Construction Time (one time)	10-30 minutes	Setting up cores; trays
	Field Time (per transect)	30-60 minutes	----
	Laboratory Time (per transect)	> 60 minutes	Seed germination checks in the greenhouse bi-weekly
	Post-Survey Processing / QAQC Time	30-60 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Germination success data are highly variable
	Relative Cost (equipment and supplies)	< $15	----
Survey / Data Quality	Accuracy (at a survey area level)	High	----
	Precision (at a survey area level)	Medium	----
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of seed bank germination survey protocols will yield quantitative data displayed in germinated seedlings per square meter categorized by species and nativity. These data are useful to identify the potential species composition, richness, and density of the seed bank in a given area and can be extrapolated up to habitat type. Additionally, this survey method can inform restoration planning or comparative analyses to other sites. An important consideration is that seed bank studies are ineffective at quantifying species that rely on non-seeding propagation strategies.

Objective

Information about the seed bank of a wetland may be a better predictor of successful wetland functioning than the presence of adult plants (i.e., plant canopy) alone because the presence of a viable and diverse seed bank indicates recent well-functioning ecological and hydrological dynamics of the site (Johnston et al. 2011). Soil seed banks also forecast subsequent adult plant species richness under optimal conditions (S. Anderson, unpublished data). However, it should be noted that a limitation of this method is that it excludes species that do not rely on seeds to propagate.

Specific objectives of seed bank surveys include:

1. Define relative species richness of germinated plant seedlings by habitat type (Figure 1);
2. Determine the potential for future recruitment of plant species within habitat types;
3. Comparison of native and non-native seed banks;
4. Evaluate potential species recruitment / propagation at a transect level under ideal conditions.

Figure 1. Labeled seed bank core with germinated pickleweed.

Equipment

Equipment and supplies needed for this survey include:

1. GPS with transect locations
2. Digital camera or smartphone with camera application
3. Core (10 cm deep and 8 cm diameter); *Helpful hint: several brands of soup cans are the appropriate dimensions and may be used as a cheap core alternative. They should be replaced when the edges dull and start bending* (Figure 2).
4. Plunger; this consists of a plug or disk the size of the core and an attached handle (several options are available at Home Depot; Figure 2).
5. Hand gardening trawl
6. Steam sterilized soil (e.g., Supersoil ®) or sterilized playground sand
7. Bucket for soil

Figure 2. Seed bank equipment.

8. Greenhouse tray / flat (useful to transport cores from the field and keep them well-organized) capable of holding 20 of the 4" pots (Figure 2). Alternatively, a small, heavy-duty plastic cement mixing trough will also work.
9. 4" nursery pots (Figure 2)
10. Permanent ink pen and duct tape or a paint pen to label the pots
11. Large, shallow tubs (called "masonry mixing tubs" at Home Depot) if automated, misting sprinkler arrays are unavailable.
12. Greenhouse space with a freshwater source
13. Greenhouse datasheets (Appendix 3.4B)
14. Vegetation species ID guide; *Helpful hint: having a seedling guide is recommended as many species look different than adult plants when they have just germinated*.
15. Hand counter (optional)

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed.

Field Methods

Soil cores should be collected at ten equally spaced points along a subset of the 25 m vegetation transects (see Vegetation Cover SOP 2013). For a habitat-level assessment, survey a minimum of three transects per habitat. Additionally, transects along high tide (wrack) lines or the edges of salt pans are recommended to characterize the areas of highest biodiversity, as most wetland plant species have positively buoyant seeds (S. Anderson, unpublished data) and will accumulate at hydrological discontinuities such as wracklines and channel bends. Soil cores should be collected during fall (October – December), soon after the first rains of the wet season to capture the seed bank at its peak (S. Anderson, unpublished data).

Specific protocols are as follows:

1. Use the duct tape and permanent pen or paint pen to label every nursery pot with the transect number, replicate number (i.e., 1-10), and date collected.
2. Use the core to collect ten individual cylinders of soil approximately 10 cm deep and 8 cm in diameter from each transect (approximately 2.5 m apart). While 10 cm depths are the goal, shallower cores are acceptable, but should never be less than 5 cm in depth.
3. The soil plug must be extracted from the corer by pushing upwards with the plunger from the bottom. Most seeds are in the upper few millimeters of the soil surface, so care must be taken to avoid pushing seeds into and/or burying seeds under the surface layers of soil.
4. Immediately following collection, place each individual core in a 4" nursery pot filled with approximately one-third steam sterilized soil on the bottom such that the uppermost surface of the core is approximately 0.5 cm below the lip of the pot. Carefully fill excess space around the corners of the pot with sterilized soil so the core cannot shift within the pot. Cores must always maintain their original orientation, with the uppermost soil surface oriented towards the top.

5. Place the nursery pots on the tray for transport to the greenhouse. Take care to position them so that they will not spill upon movement and will retain the original orientation of the soil plug throughout the collection and transport process.
6. Should you need to carry the cores over a great distance to return to your vehicle or if your drive back to the laboratory is over rough ground, it is recommended to sprinkle a light dusting of steam sterilized soil over the surfaces of all seed cores. This should minimize any potential for seeds to "disperse" from one core to another due to unexpected jarring. This light soil covering should generally be washed away with the first irrigation.
7. It is also essential to create at least one "control core" per transect. A control core consists of a pot filled only with sterilized soil (to the same overall height as the seed core pots), and appropriately labeled as "control" with the site and date. These controls should serve to detect contamination, either during transportation or (more commonly) in the greenhouse.

Greenhouse Methods

1. Transport soil cores to a greenhouse, taking care to minimize bumps and disturbances to the cores. While a formal greenhouse is not technically required for this procedure, it is essential to have a location that is well lit and slightly warmer than ambient winter conditions to promote rapid germination/growth. Consistency and control over ambient conditions is recommended (e.g., light, heat). There has been success using plastic-covered sheeted areas in well-lit sun rooms at ranger stations/remote sites. Wherever is chosen, it is essential that the the germination location is sealed from external seed sources and wind (to avoid contamination of the cores with ambient seeds).
2. Saturate all the cores with fresh water and make sure cores are watered routinely. Two protocols have been used with similar success. Given available infrastructure, one or the other is likely preferable, but should be applied consistently for each individual project, and if repeated over time.
    a. Option 1: maintain cores in a large tub (Figure 3A) and fill the tub with approximately 1-2 cm of freshwater daily. Water should wick through the bottom holes of the pots and keep the soil moist. If using this method, you must change the pooled water in the trays once every other week (maintaining approximately 1-2 cm of water throughout the growth period) and mist or spray the cores with fresh water several times a week (once daily is recommended).
    b. Option 2: mist cores heavily (5-10 minutes) twice a day from overhead sprinklers.
3. Germinated seedlings (Figure 3b) should be counted, identified, and photographed every two weeks for up to three months or until all seedlings are identifiable (e.g., flowering). Many species should germinate quickly and be identifiable within the first several weeks of watering. However, the grasses will be difficult to identify until they have flowered/formed seeds. This may take as long as three months. A core that has no germinated seeds after six weeks may be discarded and scored as zero seedlings. Control cores should be maintained for the duration of the seedling census.
4. Record all counts of species on the greenhouse datasheets (Appendix 3.4B).

Figure 3. Photos of a collection of cores arranged in a watering tub (A) and an individual potted core (B) in a greenhouse.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 3.4B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

The seed bank protocol requires extensive QAQC procedures to be completed before the data are analyzed. After each greenhouse count has been completed and photographs of all cores have been taken, the lab technician lead should take reference photographs of each species identified. The QA Officer should verify the identification of each species for each count based on these photos and reference materials. Additionally, after every other greenhouse count, it is recommended that photos be taken for each soil core sample from both an oblique overview (including transect number on side of pot; Figure 1) and direct overview (Figure 3b) for future count verification and additional QAQC against the greenhouse datasheet (Appendix 3.4B).

Office QAQC procedures should include creating a spreadsheet tab for each counting event, comparing the species tallies for each count (comparing the tabs of the spreadsheet), locating quantity discrepancies between counting events (referencing previous counts), and verifying against past soil core and voucher photographs. This step should be completed by the QA Officer and should involve cross-checking between the tabs, photographs, and datasheets for each count. If germinated seedlings die before the end of the experiment, they should still be counted in the final tallies for that core.

Quality Assurance and Quality Control (QAQC) procedures should then be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedures completed, data can be used in multiple analyses. Seed bank germinated seedlings should be identified to species. Cores can be analyzed by number of germinated seedlings per m^2 and averaged across each transect or habitat type. Additionally, seed bank vegetation species lists can be compared across habitats or areas.

Additional analyses for species diversity (e.g., the Shannon-Weaver Index) may be conducted.

Health and Safety Precautions

Not applicable.

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APPENDIX 3.4A

	Evaluation Metric	Seed Bank	Notes
	Correlation to L2 CRAM	Attribute 4	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Soil, nursery pots, soup cans, plunger
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	Can get heavy if cores are potted in the field
	Ease of Implementation	Easy	----
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2 +	----
	Training Requirements	None	----
	Seasonality of Survey Time	early Fall	Peak of the growing season
	Suggested Frequency	Annual	----
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	No	----
	Availability of Online / External Resources	Some	----
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	High Disturbance	Soil disturbance
	Vegetation Height Limitation	No Limitations	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	----
	Regional or Broad Implementation *	Infrequently Used	----
	Potential for Hazards / Risk	Low to No Risk	----
	Restrictions	Special Status Species; Cultural	Soil disturbance may be a restricted activity within some locations

** based on monitoring literature review*

APPENDIX 3.4B

Date: ______________________	Counter:	Entered:
	Recorder:	Photos Uploaded:
End Time_________ Start Time: ________	Photographer/s:	QAQC'd:

Transect	Core	Sprouts	Unk. Grass	Unk. Salicornia	SAPA	Unk. Aster	Unk. Forb (not Sali)	JACA	FRSA	CRTR	POMO	PAIN	MEIN	Cusa (?)	Other	Notes (Include No Seeds)