Salt Marsh Secrets

Who uncovered them and how?

By Joy B. Zedler

An e-book about southern California coastal wetlands for readers who want to learn while exploring

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This e-book records favorite stories about salt marsh secrets that my collaborators and I uncovered while studying southern California coastal wetlands, from the 1970s to date. In 1986, we became the Pacific Estuarine Research Lab.

Please download the files as they appear online and enjoy learning what we learned…and more. You'll meet many "detectives," and you'll be able to appreciate how they learned so much--undeterred by mud and flood. Learn while exploring the salt marshes near you!

Each chapter (1-21) is being posted at the TRNERR as a separate file (PDF).
Chapter numbers precede page numbers (for chapter 1: 1.1...1.14).
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Perennials and annuals:
Unique growth strategies!

Knowing how a perennial plant grows helps us understand the antics of annual plants. In science, we nearly always understand things better when we can compare and contrast very different organisms. First, however, let's see how researchers measure plant abundance and growth.

Pam Beare (left) counted the occurrences of perennial pickleweed in subplots of a 20x100-cm frame to describe abundance at 10x10-cm and 20x100-cm sampling scales. This nondestructive approach works for monitoring where a species occurs (its distribution). You don't have to harvest any of the plant, so you can return to the same spot again and again.

There are some shortcomings, however, because a few branches in a 10x10-cm subplot are scored the same as if there were many branches. Also, a species could grow from short to tall, without any change in presence on the data sheet. Also, when the vegetation has many species, it takes a lot of time to count the occurrences of each species in every subplot. So, this method is better for describing a monotype of pickleweed (above). It is nondestructive and easy, but not great for general use.

For widespread sampling, we used 0.25-m^2 circular plots. A real advantage is the low ratio of perimeter to area, which reduces the number of decisions a sampler has to make—is this plant "in" or "out"? It might sound odd, but there are lots of decisions to make, and you have to make fewer decisions using a circle than an oblong.

As a demonstration, calculate the perimeter of a circle and a rectangle of equal areas, for example, a circle, compared to a square and an oblong, all the same area, such as 1 m^2.

Like many ecologists, we estimated cover to assess abundance (how much is there). Cover means the shade that a species would cast on the soil surface at high noon in summer. Also like others, we used cover classes (<1, 1-5, 6-25, 26-50, 51-75, and 76-100% of the area of the plot being sampled). Some ecologists attempt to estimate cover percentages to the nearest 5%, but my eyes are not that consistent! The 6 cover classes are fairly precise for plants with little cover, which is useful when you're interested in rarity. Estimates do vary among estimators, however—one person might tend to estimate higher than another. So "calibration" among samplers is useful! In summary, estimating cover class is rapid and repeatable, with training.

Below, we were sampling cordgrass along the water's edge, using a transect line and the line-intercept method. We counted the number of decimeters in every meter that intercepted at least one cordgrass leaf. This is another quick way to measure cover.

But there's still the issue of growth. A canopy (cordgrass for example) could have 76-100% cover year-round, while its height increases from 10 to 100 cm during the growing season.

Many ecologists combine a measure of canopy height with the estimation of cover class. The combination is a reasonable nondestructive sampling approach for species abundances and growth. But height is just one form of growth; what about increased stem density and weight?

There's a much better method to compare species' growth, but, as the name indicates, the sampling is destructive. The harvest method requires clipping the biomass at ground level and removing it. It is bagged in the field and taken to a lab for drying and weighing. This is the most common method of measuring plant growth. It works best for plants that die to the ground in winter, like cordgrass, so that all the green biomass produced during the growing season is that year's productivity. Most of the other perennials in the salt marsh also die aboveground and remain dormant as roots and rhizomes underground. There's an important exception….

The harvest method doesn't work so well for perennial pickleweed, which has a lot of green biomass year-round. It's hard to know which biomass was produced in the current year.

Dr. Chris Onuf conducted the most thorough study ever of perennial pickleweed growth. He used two methods in Mugu Lagoon's large areas of monotypic perennial pickleweed. For the harvest method (25x25-cm plots), he clipped plants from new plots every month. With data on dry weight, he calculated month-to-month increases. And by adding the increases over a year's time, he tried to estimate annual biomass production. He was unsatisfied with the results, however, because the harvest method underestimates (results are too low) for a species that loses lots of branches between sampling periods. Large loss of branches = large error in estimating productivity. Also, his biomass data were highly variable, both across the marsh and over the year. He was concerned that low average biomass might be due to patchy vegetation, rather than low productivity. So, while the harvest method can be better at measuring growth than cover and height, harvests do not track lost branches or patchy biomass.

Chris devised a new but tedious method to account for losses and gains in branches. Month after month, Chris tagged individual branches and measured changes in the numbers of branches and the length of branches beyond his tags. That way, he could record both branch elongation and mortality. Then, using equations to convert branch elongation to biomass, he could compare results of the harvest and tagging methods. Perennial pickleweed gradually gained and lost branches so the total biomass stayed relatively constant, as indicated with the harvest method. This supports the "year-long growing season" described earlier for southern CA salt marshes.

- Perennial pickleweed slows down in winter, but its growing season never ends. The plant's branches stay green year-round and the root system and many branches live for many years. Older branches are replaced by younger branches, and the same is probably true for the roots belowground. In winter, it probably photosynthesizes ("fixes carbon" to make organic matter) just enough to compensate for respiration ("burning" organic matter to supply energy for its millions of cells).
- Most salt marsh plants are "summer active;" their growth increases in March when the tides aren't very high and the sun warms the marsh and provides more light for photosynthesis. They reach peak biomass in September or October.
- In contrast, box thorn (chapter nine) grows in the transition from high marsh to upland and is "winter active" like coastal sage scrub plants. It produces leaves during the wet season, drops its leaves in summer and goes dormant till the next rainy period.

Now let's compare annuals to salt marsh perennials

Perennial pickleweed is evergreen; annuals are not. Perennial pickleweed produces new branches nearly every month; annuals don't do that! Cordgrass can grow a meter tall from March to August; most annuals can't do that. Cordgrass reproduces by rhizomes; annuals can't do that.

When the seeds of perennial plants germinate after a rainfall, they invest more of their energy growing roots deep into the soil, so they can survive long after the surface soil has dried. When they invest heavily in roots, they have less energy to invest in shoots. Annuals don't do that. Annuals produce enough roots to grow enough leaves to capture enough energy to flower and fruit. When conditions are suitable, annual plants show a burst of growth after seeds germinate, then quickly flower and fruit, and then die--all within a growing season. Their seeds go dormant until the next time conditions are suitable for germination.

Where can I watch annual plants grow?

After the first rainfall that follows a dry spell, look carefully along sidewalks and in cracks in the streets. You should see seedlings popping up (growing). Many of these seedlings will be "annuals"—that is, species whose many seeds germinate and die, leaving only seeds for the next rain event. That's a short time to complete an entire life cycle or generation, but it is a very effective strategy in Mediterranean-type climates, where there is little soil moisture between rainfall events.

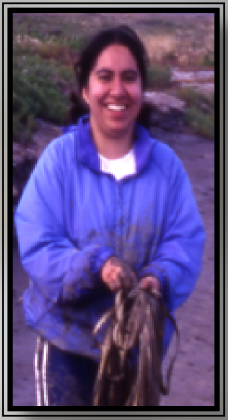
The high salt marsh has many annuals at its upper edge, between the high marsh and salt pans (also spelled "salt pannes"). As the name "pan" implies, these are flat areas that accumulate so much salt at the soil surface that few plants can grow there. Large areas are bare (unvegetated). They appear white during dry spells. Below are photos of a pan after rainfall (left), the edge of a perennial pickleweed clone with drying mud between summer high tides (middle), and annual pickleweed colonizing the same mudflat (right). During wet weather, the salt can wash away, and the seeds of annual plants can germinate. While there are many species of annuals that can colonize the salt pans, the salt marsh plain has just one annual plant, annual pickleweed.

Why do you think the high marsh edge supports several annual plants while the marsh plain has only one? In both places, seeds need light, but large bare areas with no plants are not limited by light. Instead, they are limited by too much or too little water and too much salt.

Intertidal pools that are >10 cm deep (see chapter eight) are too wet for plants, both perennial and annual. Few annuals can grow well in soil that is nearly always waterlogged (filled with water and lacking oxygen). In the photo above, the cracked soil indicates that there was a pool during high tide. The intermittent pool is probably bare because it is too wet during high tide. During a series of low tides, it will probably be too dry! Salt pans are different; they develop above the typical high tide and are almost always too dry and too salty for seeds to germinate.

Of course, seeds have to be present for annuals to pop up, and it's hard to tell if they are. Most seeds are tiny and covered by mud.

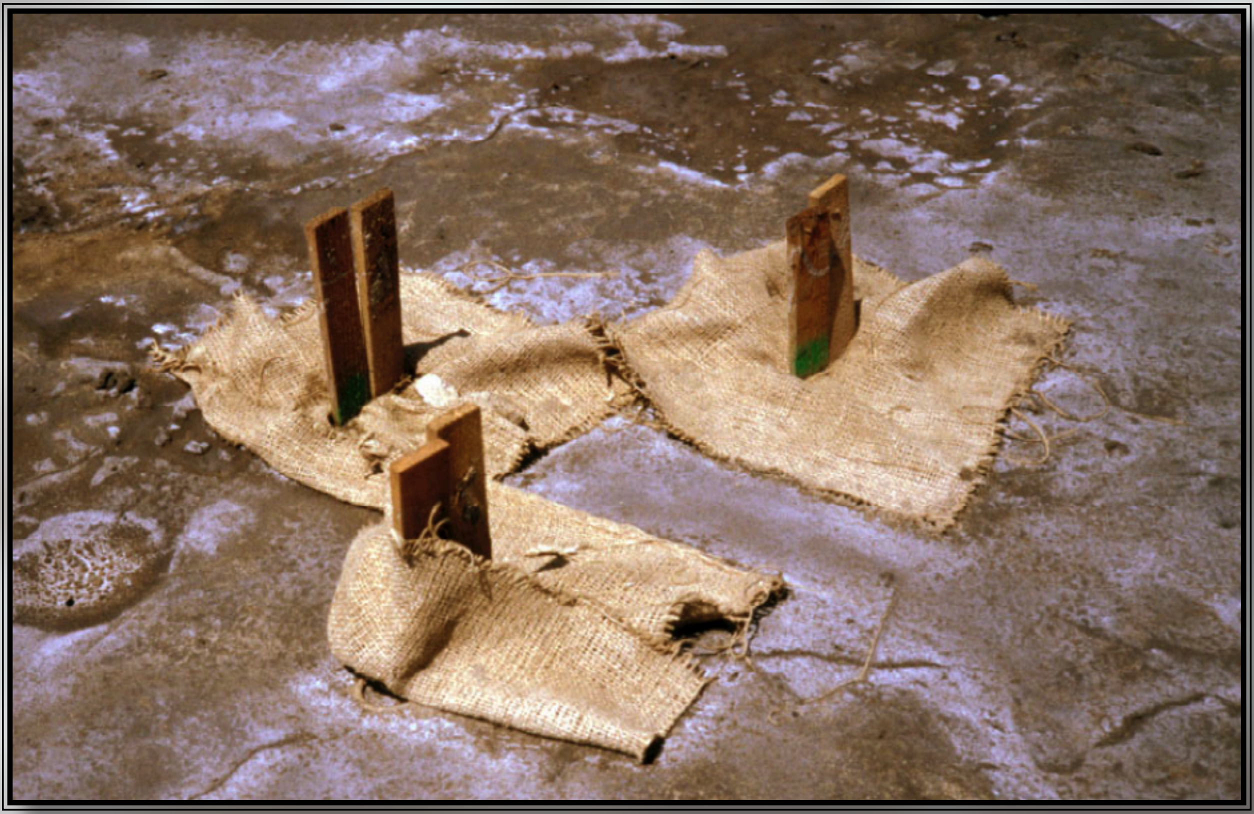
How can we tell if seeds are present? We can collect soil samples to test for a "seed bank" (seeds that are present in the soil). And if no seeds germinate (no seed bank), then how can we tell if seeds would germinate if they washed in or blew into the salt flat? The 20-acre Model Marsh turned into a salt flat after it was opened to tidal flow in February 2000. We knew there was no seed bank, because the excavated marsh plain had been covered with sediment for over a century. The excavation uncovered some evidence of use by Native Americans, and a thorough anthropological survey failed to reveal any significant relicts. So the new marsh plain lacked both seeds and, during February-April, it also lacked sufficient tidal inundation. We didn't know how dry it was, so we added seeds that we knew were viable (alive), to see if they would germinate.



As part of her doctoral research, Hem Nalini Morzaria-Luna added viable halophyte seeds to the large salt flat that formed in the newly-excavated marsh plain at Tijuana Estuary's Model Marsh. She also added squares of burlap to keep the seeds from moving and vertical shingles to provide some shade. When nothing germinated, she knew that:

- Seeds, light, and shade were not limiting seedling establishment.
- Hypersaline sediment was the most likely limiting factor.

The photo below was taken on 29 April 2000. It took many months of tidal flushing to lower soil salinity enough for vegetation to cover this marsh plain.



Species	Planted	Recruits
Perennial pickleweed	840	17,703
Annual pickleweed	735	15,978
Sea blite	795	1,668
Arrow grass	810	73
Salt marsh daisy	840	33
Salt wort	720	26
Sea lavender	795	24
Alkali heath	765	2

Contrast the bare salt flat at the Model Marsh with the "recruits" (seedlings) that we counted in the Tidal Linkage (on left; Zedler et al. 2005).

We had planted similar numbers of each of these 8 marsh plain species in April 1997, but only 3 species recruited in large numbers: the perennial and annual pickleweeds, and the short-lived sea blite.

Although no two plant species are identical, most halophyte seeds tend to germinate where there is plenty of light, not too much water, not too little water and not too much salt.

How much salt is too much? It depends on the species. Both annual pickleweed and perennial pickleweed seeds can germinate in seawater (3.4% salt)! That helps explain why pickleweeds are widespread and abundant in fully tidal salt marshes. How did we learn that? By testing about 100 seeds per species using water of different salinities (usually 0, 0.5, 1.0, 2.0, and 4.0 % NaCl).

Annual pickleweed had other secrets that allow it to co-exist with all the perennials on the marsh plain. I counted seeds on several plants and found an average of 80 seeds per plant! Because annual pickleweed needs to grow from seed every year, it needs to produce a lot of seeds so that at least one per plant (on average) can replace the parent that dies.

Very few halophytes have seeds that can germinate in saline water and seedlings that can establish in saline soil. Although annual pickleweed is the only native annual on the southern California salt marsh plain, a few others sometimes establish temporarily during years of heavy rainfall and flooding when the salts are leached out of the soil.

Annual pickleweed is rare or absent in non-tidal lagoons, but it persists in fully-tidal salt marshes. It even bounced back, slowly, from the 1984 hypersaline drought at Tijuana Estuary, when we almost lost that population. Its slow recovery and its absence in lagoons indicate a short-lived seed bank. That is, the seeds probably do not remain viable more than a year or two. Long-term field observations helped us understand its limitations, and careful experiments taught us a lot more about the abilities of this hardy, yet vulnerable, annual plant.

Annual pickleweed seedlings seemed to be restricted to shallow pools, while perennial pickleweed established across the Model Marsh in Tijuana Estuary. Why? Alison Varty found that annual pickleweed was outgrown by perennial pickleweed except in shallow depressions. Perennial pickleweed could not tolerate as much waterlogging as the annual. And what a surprise to learn that a tiny difference in pool depth was enough to tip the balance in favor of the annual.

How did she figure out how deep a pool would have to be to exclude the perennial but not the annual? First, we hypothesized that annual plants that coexist among perennial dominants might persist in microsites that are more stressful to their competitors—a bit like a home-town advantage! We expected the annual to persist in waterlogged depressions, but not the perennial.

(Photo by Varty)

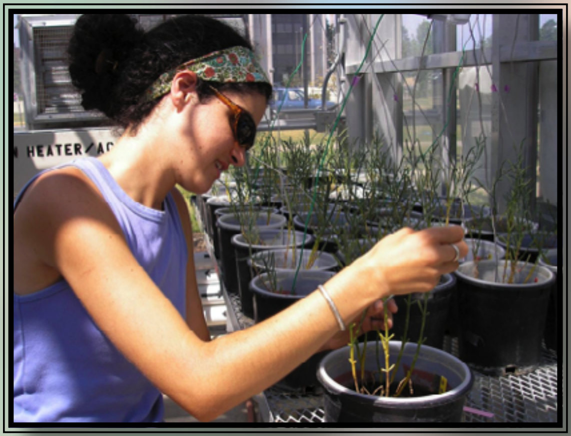
Alison created depressions that were 5 cm and 10 cm deep, with and without clipping the perennial pickleweed (removing all shoots).

With help, she used a 0.25-m^2 corer and temporarily removed circular blocks of perennial pickleweed sod plus 5 or 10 cm of underlying sediment (discarded). Then the team replaced the sod to achieve the desired depressions. She added annual pickleweed (both as seeds and as 4 seedlings) to all experimental plots.

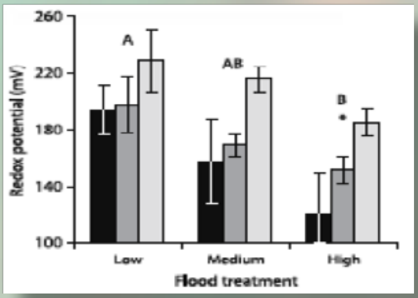
Control plots (no depressions) had the corer pushed into the soil 15 cm and removed. Note that a control plot should only differ in the factor being tested, so ours had roots cut and soil disturbed, but no change in elevation.

Alison waited, checking plots at 2 and 4 months. The 10-cm depressions decreased the cover of the perennial by about 30% compared to control plots.

In 5-cm depressions, the annual grew taller and produced more flowers, completing its life cycle. Experimentally reducing the perennial's canopy cover in shallow depressions increased the annual's survival.



(Photo of Varty by Bonin)



And now the important cause → effect question: How did the annual tolerate more waterlogging? In a greenhouse experiment, Alison measured the amount of oxidation (to indicate oxygen release from roots and tolerance of anoxic soil—recall that root cells need oxygen). She measured oxidation with a redox (reduction-oxidation) meter attached to redox probes inserted into the soil. As we suspected, probes into the annual's soil showed greater oxygen release from roots than probes placed with the perennial! Another secret revealed. The results for perennial pickleweed (black bars), annual pickleweed (with bars) and mixed plantings (gray bars) show that the soil in the pots with annuals always had higher redox (more oxygen) than the perennial. Also, the pots with high flooding had lower redox (less oxygen) than with low flooding. It all made sense! (graph from Varty and Zedler 2008). It all made sense!

We concluded that waterlogged microsites give the annual an advantage among dominant perennials. The regionally rare annual pickleweed could use shallow depressions on restored marsh plains.

Kathy Boyer surprised me and many ecologists when she showed that annual pickleweed could outgrow our perennial cordgrass when fertilized with nitrogen! Under normal conditions, cordgrass grows taller and produces much more biomass than the annual pickleweed. But when she fertilized her experimental plots in Sweetwater Marsh, San Diego Bay, with urea (N source), the annual outcompeted the perennial grass.

Why is that such a surprise? Actually, it was a double surprise, because the two species differ in life span (perennials typically outcompete annuals by growing taller stems and more extensive roots), and because pickleweed is a "C-3" plant while cordgrass is a "C-4" plant.

C-4 plants produce a 4-carbon chain during photosynthesis, and C-3 plants produce 3-carbon chains during photosynthesis. That doesn't explain much, unless you are a plant physiologist. What ecologists get excited about are the adaptations that are associated with C-3 and C-4 carbon chains. C-4 plants are notably highly productive--usually much more productive than C-3 plants. The annual pickleweed's secret was its potential to grow tall given adequate supplies of nitrogen. Thanks to Kathy for suggesting (and implementing) a field experiment to test her hypothesis that annual pickleweed was replacing cordgrass in parts of our study site (Boyer and Zedler 1999)!

We had good reasons to expect a C-4 perennial to outgrow a C-3 annual. Yet Kathy saw the opposite pattern where she was adding N to cordgrass plots. It's not enough to see a pattern; a researcher has to test the effect of N addition. So, we set up a field experiment to test the effect of nitrogen on competitive outcomes. As a result, Kathy revealed another of annual pickleweed's secrets! It could, given plenty of N, grow very tall and accumulate more biomass than cordgrass.

I saw another pattern in the Model Marsh: Annual pickleweed seedlings were not very dense, but some individuals became very robust. The mats with cracks and rolled-up edges are bluegreen algae (cyanobacteria), which "fix" nitrogen. Fixing N means converting N_2 gas in the air into usable molecules, like ammonium, NH_4, which plant roots can absorb.

Do you think it's a good hypothesis that N supplied by bluegreen algae can make the annual more robust and able to produce many more than 80 seeds per plant? I do.

Which annuals occupy the high marsh edge?

Is there a specific time that stimulates germination so seedlings can establish? Seawater rarely inundates the soil at the upper edge of the high marsh. Compared to the marsh plain, there is less salt inflow, but there's also less opportunity for salt to flow out with the tide—tidewater that doesn't flow in doesn't flow out. But when the occasional high tide does reach the dry soil of the high marsh, it soaks into the soil quickly. Then, after the occasional high tide recedes, the sun dries the soil and the salt gets concentrated. So a little salt influx can still result in a lot of salt, that is, extremely hypersaline conditions that prevent germination.

But suppose there are 1-2 days of heavy rain during spring that coincides (occurs at the same time) with a period of neap tides, when the high marsh is mainly wetted by freshwater (rain). The freshwater can dilute the soil salt a little more each day when there is no high tide to replace salt. There's a short period with moist soil of low salinity when seeds could germinate. Voila!

Photo of goldfields (*Lasthenia glabrata*) at Los Peñasquitos Lagoon, by Greg Noe

Greg Noe called it a "germination window." Annual species take advantage of the brief germination window at the salt marsh-upland edge in early spring. Walk along the upper edge of the salt marsh in spring after substantial rainfall and look for newly germinated seedlings. You might have to bend waaaaay way down to spot them, as some are only an inch or two tall. Why was it, then, that one of our tallest researchers spent endless hours on his knees uncovering their secrets--what makes them germinate when and where they do? Answer: His endless curiosity!

Greg wanted to know everything about everything when he arrived at SDSU. Of all the secrets he might uncover over the course of his joint (with UC-Davis) doctoral program, he chose to investigate salt marsh annual species. No one else had studied them in detail. For most people, they were too small and too short-lived to attract much attention. They were ephemeral.

- The term "annual" might be confusing; it means that every year the species has to produce new plants from seed. "Ephemerals" (meaning short-lived) are annual plants that germinate, grow, reproduce, and die, all within a few weeks or months.
- There are also biennials, which grow roots and shoots the first year and then flower the second year, then die.
- Perennials are plants that live longer than two years, including trees that can, if allowed, live hundreds of years.
- Annuals are usually much smaller than perennials, and they usually have shallow roots and soft leaves. Perennials take time to grow deep roots and coat their leaves with water-resistant layers. Such leaves resist wilting and allow perennials to survive periods of low moisture by reducing the loss of internal water..
- Ephemerals can avoid some of the stresses of hot sunlight and dry soil by germinating during and after rainfall events that lower the soil salinity and wet the soil enough to stay moist for 3-4 weeks. To avoid drought, ephemerals produce less shoot biomass than perennials, so they don't require extensive roots. Because ephemerals use less energy growing roots and shoots, they can invest more energy in flowers and seeds. They flower and reproduce as soon as possible, before the soil dries and concentrates salt.

Many ephemeral plants only survive for a short portion of the growing season.

- What is a growing season? In Wisconsin, it's a simple concept—it's when you can grow corn, about 120 long, warm days. The rest of the year's 245 days are too cold and have too few hours of daylight to grow crops. Winter is a good time to be dormant!
- Because the Pacific Coast of California has no frost or snow cover, many plants can grow in both winter and summer. "winter" is the season when rainfall is most likely to occur.

Greg Noe began his work with a field study of soil moisture, soil salinity, and seed germination in high salt marshes of Tijuana Estuary, Sweetwater Marsh, and Los Peñasquitos Lagoon (see map, chapter one). He sampled Sweetwater Marsh weekly plus daily for 5 days after every rainfall during 1996-1997, followed by irregular sampling in 1997-1998. Later, he demonstrated that daily sampling allowed him to capture the low-salinity/high-moisture conditions that trigger germination. If he had sampled weekly or monthly, he would have missed critical times that allowed seeds to germinate and produce seedlings. Greg's curiosity paid off!

- When you're the first to conduct research on something that changes with time, you won't know how often to sample. So, it pays to sample very frequently. Later, you can decrease sampling frequency if the early data indicate periods of constant or slowly-changing conditions.

* Note that the National Weather Service reports rainfall daily. Their long-term records allow us to calculate averages, but very few rainfall events are "average"—instead, many are very low (barely measurable or "trace") and many are extreme.

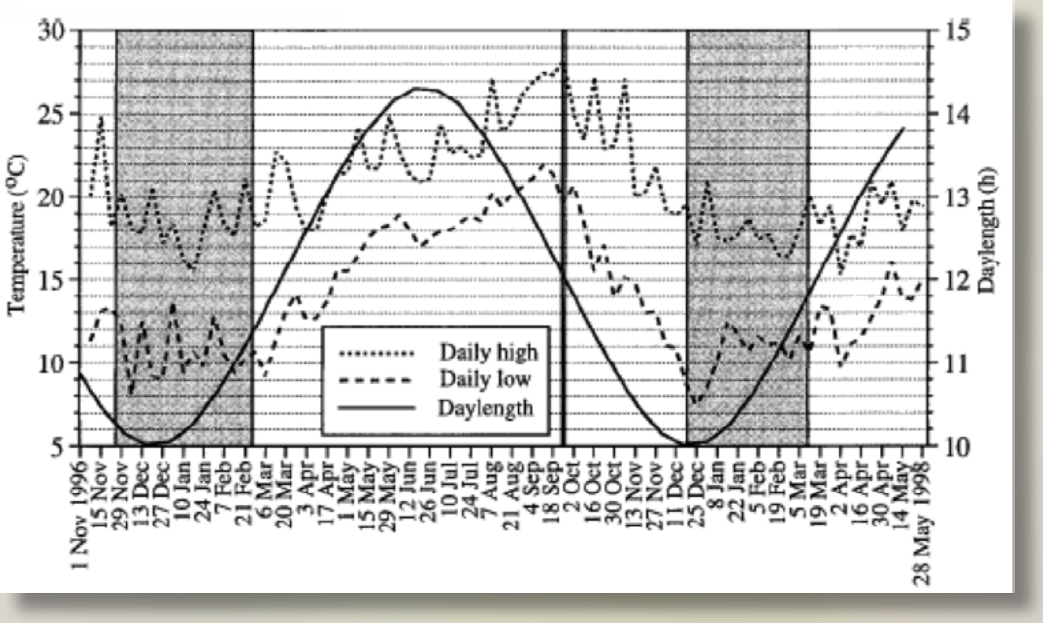
Greg found that rainfalls greater than 3.0 cm in a single day were enough to increase soil moisture and decrease salinity and trigger seed germination. A 3-cm event might not sound like a lot, but when a storm comes along, it often pours! Greg analyzed rainfall records from 1850 to 1997 and found a range of 9-66 cm rainfall per rainyear during those 147 years. Do you remember any rainyears with as little as 9 cm of rain?

As illustrated in chapter two, a rainyear covers the wet season by summing rainfall from July 1 through June 30. This makes sense for southern California, where upland vegetation grows most actively during the wetter months.

Germination can follow rainfall, but it can also follow freshwater flooding. It takes a lot of runoff to flood the high salt marsh. When it happens, the freshwater from rivers can also cause seeds to germinate—at least the seeds that are not washed away.

- Meteorologists can correlate rainfall with flooding, especially if they add up rainfall for the entire watershed (using a model that estimates rainfall between weather stations). Other models show how much of the upstream rainfall is trapped in lakes or reservoirs upstream and how much flows downstream.
- Runoff is not just the result of rainfall and reservoirs, however; it also depends on the condition of the soil when it rains. If the soil is already saturated, much more water will run off than if the soil is dry and some infiltrates. Infiltration also varies, being greater with slow rainfall than with rapid, large, pounding rain drops.
- The vegetation makes a difference, too, by intercepting water and reducing its impact. Notice the effect of a tree after rain stops in the open—when you walk under the tree, it keeps dripping water, and the water falls more softly than out in the open during rain.

Over two winters, Greg's results were very clear: Seeds germinated when soil moisture was high (>40%) and salinity was low (< 10-35 ppt) after salts were diluted and leached out of the soil. The germination window for the high marsh ephemerals (below) specified the effects of both soil salinity and moisture. Earlier I had used the term "low-salinity window," which failed to recognize the effect of soil moisture. Bravo, Greg!



Greg plotted the daily high and low temperatures from the San Diego airport weather station (Lindbergh Field) along with daylength (from Noe and Zedler 2000).

How long was each germination window (shaded area)? How were these two rainyears different? Greg's samples of salt marsh edges at three locations (weekly in 1996 and monthly in 1997) helped him characterize patterns among species and microhabitats.

More secrets were revealed: Some seedlings established early in the germination window; others germinated late in the germination window, and some had prolonged germination (throughout the germination window). In fewer words, Greg found individual responses to environmental cues.

Saltmarsh bird's beak in flower within a salt pan; photo by Greg Noe

The 13 species with the most dense seedlings in the field in 1996-7 were highly variable in abundance and timing. For example, rabbitsfoot grass was #7 in abundance in 1996 and absent in 1997, along with brass buttons and rye grass. Absences were caused by flooding, sedimentation, and burial of the highmarsh at Los Peñasqitos Lagoon! Of the 6 species that had prolonged germination in at least one of the two years, only one had the same timing in *both* years! On the other hand, 6 of the 7 exotic species listed here had the same pattern, namely germinating early in 1996 but absent in 1997. It would be nice to know which factors kept exotic species from germinating in 1997! Here is a summary of Greg's data for 13 species:

Species (Family), exotic (not native)	**1996**	**1997**	**Density rank**
Pineappleweed, *Amblyopappus pusillus* (Asteraceae)	prolonged	early	4
Bird's beak, *Cordylanthus maritimus* ssp. *maritimus* (Scrophulariace)	late	late	12
Brass buttons, *Cotula coronopifolia* (Asteraceae); exotic	early	absent	9
Hutchinsia, *Hutchinsia procumbens* (Brassicaceae)	prolonged	early	5
Toad rush, *Juncus bufonius* (Juncaceae)	late	prolonged	3
Goldfields, *Lasthenia glabrata* ssp. *coulteri* (Asteraceae)	early	prolonged	6
Ryegrass, *Lolium multiflorum* (Poaceae), exotic	early	absent	11
Hyssop loosestrife, *Lythrum hyssopifolium* (Lythraceae), exotic	early	absent	8
Ice plant, *Mesembryanthemum nodiflorum* (Aizoaceae), exotic	prolonged	early	2
Sickle grass, *Parapholis incurva* (Poaceae), exotic	prolonged	prolonged	1
Rabbitsfoot grass, *Polypogon monspeliensis* (Poaceae), exotic	early	absent	7
Sow thistle, *Sonchus oleraceus* (Asteraceae), exotic	late	early	13
Salt sandspurry, *Spergularia marina* (Caryophyllaceae)	late	absent	10

What were the germination cues? Some species might respond to photoperiod, which is the length of sunlight per day. Some might respond to specific temperature or moisture levels. Read on to see how he devised experiments to identify germination cues….

After observing the diversity high-marsh ephemerals (up to 20 species) and their various establishment patterns, Greg selected 10 that produced enough seed for experimentation in a growth chamber (a refrigerator-size incubator where he could control light and temperature) to test factors alone and in combination.

Seeds trying to germinate in greenhouse microcosms; photo by Greg Noe

The ten species were:

Pineapple weed (native, common)
Salt marsh bird's beak *(native, rare)*
Brass buttons_(exotic, common)
Hutchinsia (native, common)
Goldfields (native, locally abundant,
 regionally rare)
Hyssop loosestrife (exotic, common)
Ice plant (exotic, common)
Sickle grass (exotic, common)
Rabbitsfoot grass (exotic, common)
Salt sandspurry (native, common)

Later experiments were set up in our greenhouse, where daylength varied naturally and where he had room for more experimental containers. But the first greenhouse experiment did not result in the temporally-varying soil salinity and moisture conditions that Greg wanted to test.

Time out! Greg realized that he would have to re-set the entire experiment. That meant counting 33,000 seeds into groups of 25 per species per pot, all over again. But first, he had to revise his microcosms so they would achieve the desired changes in salinity and moisture. What a trooper!

I have often said that the most important attribute one needs to achieve a PhD is determination. Greg had more than enough. But that doesn't mean it was fun to re-do the experiment. In the end, it was all worth the ordeal and the satisfaction of a job well done (see Noe 1999 and Noe 2003).

Greg's overall hypothesis was that abiotic (non-biological, i.e., physical and chemical) factors differentially affect the germination of salt marsh plants. "Differentially" means that the same factor affects different species in different ways. To test that hypothesis, he compared germination responses of native and exotic species and common and rare species, all while varying temperature, photoperiod, soil salinity, and soil moisture.

After weeks of preparation and incubation in the growth chamber, the seeds revealed many secrets. Soil salinity, soil moisture, temperature, and photoperiod each affected the percent of seeds that germinated and the speed of germination.

- In general, soil salinity had the largest effect on species; more species responded, and the magnitudes of the responses were larger, for soil salinity than for the other abiotic factors.
- Also, salinity had a stronger effect when moisture was low, and moisture had a greater effect when salinity was high.
- However, salinity was not the most important factor for all species tested. Greg was able to explain why some species cannot germinate outside the cool-season germination window and why the following species could:

Photo by Greg Noe

- Non-seasonal germination by Hutchinsia, hyssop loosestrife, sickle grass, and possibly goldfields occurred in response to salinity, temperature, and photoperiod.

How did Greg decide what to control and how to control it? These were not easy decisions!

First decision: Which variables to control? Greg worked from his field observations and other studies in the literature to choose salinity, moisture, temperature, and photoperiod.

* To identify all their individual and combined effects, would take 960 treatments (10 species x 4 factors x 4x3x2 pairs, i.e., combinations of two at a time) plus replication (4 pots per treatment), for a total of 3,840 pots. That's a lot of pots if you measure each one 10 times per month (38,840 counts). What would you do?

Greg opted to explore salinity and moisture effects for 7 species and temperature and photoperiod effects for 8 species, creating a more manageable experiment. He could run the two experiments in tandem, reducing the number of pots (microcosms) to be crammed into a growth chamber.

Another decision: How to define germination? Is it the number of seeds that germinate by the end of some time period, like 4 weeks? Or is it the speed at which seedlings accumulate? Greg developed a new way to characterize the "speed" of germination. He knew that salt reduced the number that germination and that salt could delay germination. Putting a number on (quantifying) that delay was yet another of Greg's contributions:

Greg's "Germination Speed Index" allowed him to characterize one more of the plants' secrets—how long seed germination is delayed by salt.

That still left variations to be explained by temperature, moisture, and photoperiod. And while those responses were very interesting and surprising, they were also rather complicated, for example:

- More hyssop loosestrife seeds germinated at cooler (November) than warmer (March) temperatures (72% at 16.7C vs. 31% at 15.3C). But look at those temperatures—they're so similar! These species could tell the difference between temperatures that were only 1.4^{o}C apart. Wow! At the same time, only the proportion of seeds, not the speed of germination, was affected.
- More goldfields and sickle grass seeds germinated at constant cool temperatures (87% and 95%, respectively) than with daily-fluctuating cool temperatures (62% and 63%, respectively).
- Germination speed was greater for sickle grass and salt sandspurry with constant cool temperature (88% and 99%, respectively) than with cool, daily-fluctuating temperatures (81% and 92%).

Greg's germination tests of ten species in the growth chamber revealed secrets that helped him explain germination by six of the species in the high marsh: Most of the effects on seeds (both the proportion germinating and germination speed) were due to salinity. That one-sentence summary doesn't include all the complications, however. Germination of the remaining four species (Hutchinsia, hyssop loosestrife, sickle grass, and possibly goldfields after a nonseasonal rainfall) could have been due to salinity, temperature, or photoperiod. Perhaps readers will be inspired to figure out why each of these four species germinate when and where they do!

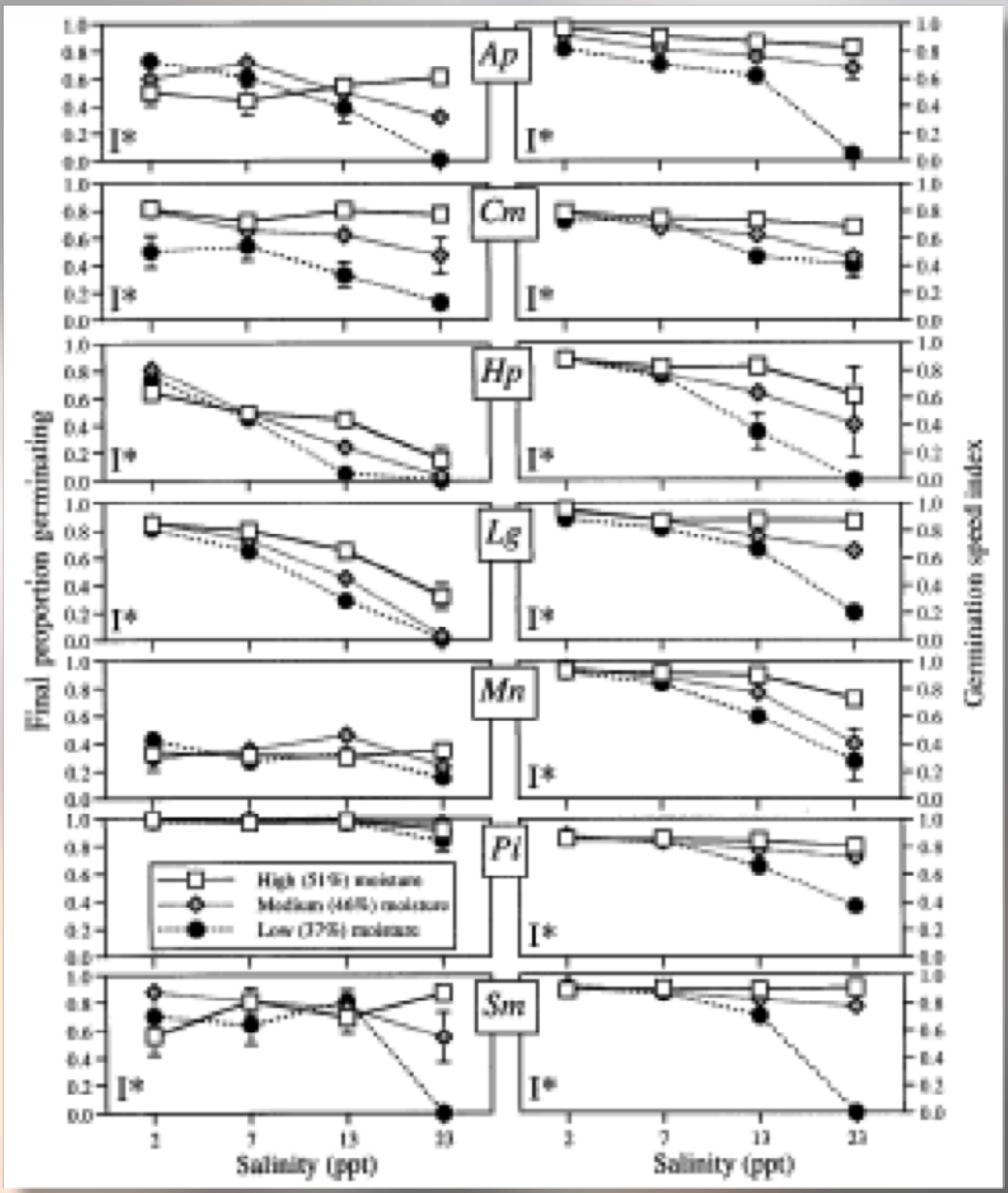
The complexity of salinity and moisture effects on germination led Greg to test the ideas that grew out of his previous studies by subjecting a mixture of seeds from 11 species (the native community identified in his previous field studies) to additional experiments. He wanted to vary the amplitude, duration and seasonal timing of soil salinity and moisture. Can you imagine how many treatments this involved and how many pots he had to monitor for germination?

- Calculate the number of pots needed for various tests: Suppose you want to compare 4 salinity levels; 3 moisture levels, each with constant vs. varying moisture. That's 4 x 3 x 2 = 24 treatments. Now multiply the treatments by the number of replicates you'd like per treatment (e.g., 4), and you get 96 pots per experiment.
- Next, experimenting with duration, Greg compared low salinity for 0, 1, 2, and 4 weeks and low and high moisture for 0, 1, 2, and 4 weeks. That's 4 x 4 = 16 more comparisons, not to mention the 4 replicates. 16 x 4 = 64 pots.

To test the effect of season, Greg tested for germination in 3 sequential experiments beginning Nov., Jan. or March. In analyzing all these results, Greg developed great statistical expertise along with ecological understanding!

As seen earlier, salinity and moisture affected germination, but the test of duration and season added understanding to species' differential responses and the nature of the response (% germination and germination speed)—each species' response was unique.

Here are the data for % germination and germination speed (from Noe and Zedler 2000).



Eight species had reduced germination when low salinity/high moisture conditions lasted only one week (compared to 4 wks). Two species responded to varying vs. constant salinity levels, but not in the same direction. One species preferred varying and one preferred constant salinity. Furthermore, the effect of variable conditions depended on the range of the temporary low salinity (0, 8, or 17 ppt). Also, the duration of the germination window (1, 2, or 4 wks) influenced % germination (for 4 species) and germination speed (for 2 species). Four species differed in % germination and 8 differed in germination speed when the "germination window" was initiated in November versus January versus March.

Another secret revealed by all this work was that the 6 exotic species were less sensitive to temporal variability than the 5 native species. Had Greg worked only with constant salinity and moisture at different levels, ignoring the variability that occurs in nature, he would have come to different conclusions!

How does a seed keep track of all these conditions? Among the 11 salt marsh species, there were differential responses both as total % germinating and the speed with which they achieved their maximum germination rate. Imagine if we knew this much about all plant species' abilities to germinate. There's a giant book, appropriately called *Seeds* (by Baskin and Baskin), but it only begins to reveal all the secrets about the seeds of earth's 200,000+ plant species.

Why did I write so much about seed germination?

First, the studies were of great use in explaining observations in the field. Second, the research showed how complicated science is and how one test with one answer leads to another question and another test. Third, the work demonstrates that, in ecology, there is always another variable that might contribute to explaining plant distributions. And fourth, this is an area of study that readers can experience and perpetuate. It doesn't take expensive equipment; it doesn't require much space, at least not for a small experiment, and the time required is manageable.

Even though you probably won't have access to a growth chamber to keep temperature and light constant, you can test salt addition and watering regimes and conditions near windows in different locations. Start by reading the methods in Noe's 2003 paper. Then get creative! Devise some experiments. Win a prize at your school's next science fair!

I suggest testing for germination cues (triggers or causal factors) for exotic annual grasses. No one will mind if you collect their seeds and dispose of them properly (not dispersing them) after your study! Don't toss them in the gutter or trash where they will end up in a land fill; instead, add a bit of bleach so they are no longer viable.

Exotic annual grasses are weeds that managers want to control. Many exotic grasses thrive in the low-salinity soils that accompany late-winter rainfall. While salt is a limiting factor for sickle grass and rabbitsfoot grass, both species expand in wet years and leave large seedbanks in the soil. The seeds lie dormant during dry years, when they appear to have left the scene. Despite appearances, the next wet year can rejuvenate the population by stimulating seed germination.

Happy experimenting!