

## 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

Zedler, Joy B. 2015. Salt Marsh Secrets: Who uncovered them and how? Tijuana River National Estuarine Research Reserve. Imperial Beach, California (http://trnerr.org/SaltMarshSecrets).

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!

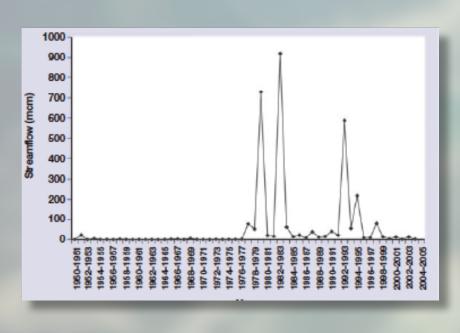
> Each chapter (1-21) is being posted at the TRNERR as a separate file (PDF). Chapter numbers precede page numbers (for chapter 1: 1,1...1,14). Layout by Emily L. Rosenthal. Photos by the author or as noted.

#### PDF name and brief description:

Preface: Learning while exploring 1DiscoveringSecrets: Introducing salt marshes 2SeasonalChange: How weather and tides change over the year 3RarePlant&Bird: An annual plant and a clapper rail 4WherePlantsGrow: The influence of salt and water and more 5Perennials#Annuals: How short- and long-lived plants get along 6SaltMarshWeeds: Which species invade and why 7Sedimentation: A little sedimentation is good; a lot is not 8Heterogeneity: Variable elevation and patchiness 9Transitions: From marsh plain to high marsh to upland 10 Testing Diversity: What diversity affects and what affects diversity 11RunoffCattailsAlgae: Freshwater pulses trigger pesky invaders 12 Dunes: Why our dunes are low and flat 13Damages: How we damage salt marshes 14GoFish: Fish and invertebrates respond to changing waters 15Animal Mobility: Mobile and immobile species 16FoodWeb: Who eats whom 17ConservationBattles: It wasn't easy saving salt marshes 18 Restoration: Returning tidal influence 19 Testing Theory: Contributions to the science of ecology 20References: References cited and other PERL research 21 PERLalumni: Where the "detectives" are now

# How does rainfall upstream cause cattail invasions and algal blooms downstream?

Sometimes a secret doesn't catch your attention until Nature reveals it again and again and again. Suppose you were busily studying a salt marsh that had very little influence from its upstream watershed--just a trickle of street runoff here and there, now and then. If there were floods in the distant past, only the "old timers" remembered them. I was a newcomer, so I focused on understanding what I could see from year to year.



Then, suppose the salt marsh's watershed and local coast experienced a series of heavy-rainfall years with much more water than the land could soak up: The floods in 1978, 1980, and 1983 offered enough experience for me to see first-hand how watersheds influence salt marshes!

These streamflow data are for Tijuana River, where it flows from Tijuana, Mexico, across the international border into the US.

The unit of measure is million cubic meters (mcm) per streamflow year. The peak flows in 1978, 1980, 1983, 1993, 1997 and 1998 were major flood years (Zedler and West 2010).

#### How do watersheds influence salt marshes?

There are two patterns:

• Salt marshes with small watersheds are not much affected by heavy rainfall—relative to saltmarshes that are downstream from large watersheds. Rainfall flowing from large watersheds downstream into Tijuana Estuary produced catastrophic flooding downstream. This pattern is spatial; salt marshes differ in flooding during a year with unusually high rainfall. Below, I'll compare Mission Bay Marsh and San Diego River Marsh, as examples. San Diego River Marsh changed substantially in 1980, while Mission Bay marsh barely noticed the flooding.

• The Pacific Decadal Oscillation (PDO) is a temporal pattern of weather that changes over decades (10-year periods), from many dry years to many wet years. The rain that falls throughout the watershed varies in amount and timing of storm events, so the amount that flows into an estuary is a better indicator of watershed effects than simply measuring rainfall near the coast. As graphed above, the streamflow records for Tijuana River showed no flooding until 1978. That was the first year in my experience that heavy rains fell throughout the watershed and caused a major flood downstream. It was the first of many years of wetter conditions and salt marsh flooding.

**EFFECT OF WATERSHED SIZE.** Two salt marshes in San Diego's Mission Bay area show how connection to a large watershed can have catastrophic flooding, while a disconnected marsh is unaffected. How could that be? The two marshes were historically one, called False Bay. The reason is the flood control channel, which disconnected the Mission Bay Marsh from its former San Diego River watershed—a 440-square-mile watershed that stretches all the way to the Cuyamaca Mountains. The channel is edged with riprap (large rock boulders). Most of the runoff flows straight to the Pacific Ocean, after leaching out salts from San Diego River Marsh.

The photo below, which was taken in a non-flood period, shows a sand bar (tan area, below BHP) near the ocean. That sand slows some river flows and pushes some tidal and river water through the spaces within the riprap. During river floods, the sand bar is eroded downstream toward the ocean. River mouths are dynamic! The channel that leads to Mission Bay also accretes sand, and that sand has to be removed by dredging in order for large boats to come and go.



www.brenthaywoodphotography.com/



interwork.sdsu.edu/fire/resources/ SanDiegoRiverWatershed.htm

The San Diego River Marsh is just above the "H" (above, left photo); its watershed stretches inland to Julian (right map). Mission Bay Marsh is barely visible on the left of the photo; its urban watershed is tiny. Most of the former False Bay wetlands have been dredged to form an aquatic park.

**EFFECTS OF FRESHWATER FLOODING.** The region's estuaries with large watersheds changed substantially during the wet years of the Pacific Decadal Oscillation (PDO). The San Diego River Marsh changed the most, because river flooding was concentrated into a narrow flood control channel. And because the San Diego River flooded repeatedly in 1980 and 1983, it was possible to watch the upstream cattail marsh and downstream salt marsh shift toward the ocean in 1980, then retreat in 1981-82, only to move downstream again in 1983. These shifts were related to changes in soil salinity, which was reduced by flooding in 1980 and in 1983 but allowed to increase with high tides in years without flooding.

Which plants invaded most extensively during the PDO's wet years? Cattails! Around the world, cattails are well known for being invasive. The species differ, and some are hybrids, but the pattern is global—cattails expand when favorable conditions (nutrients) become plentiful.

A well-known ecologist at Connecticut College, Dr. William Niering, once described cattail invasions as "the cattailization of America." He was referring to the ability of cattails (species of the genus *Typha*) to invade and dominate many marshes, so long as they were not saline. In northeastern US, he was referring to coastal salt marshes that had been diked to restrict tides and seawater influence. As rainfall and river flows gradually diluted the salts behind dikes, seeds of cattails could germinate.



The largest cattail marsh in the U.S. is Horicon Marsh in southeast Wisconsin (www.fws.gov).

Why are cattails are such aggressive invaders? There are many secrets that have been discovered and reported in the scientific literature. Here are several:



hseabrookeleckie.com

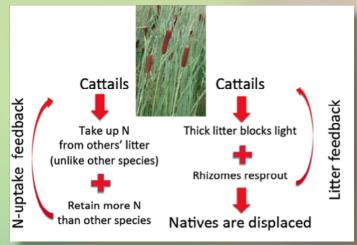
• Cattails produce lots of seeds that are easily dispersed (spread). Cattail seeds are tiny and lightweight, and they have fluffy hairs attached so they can float with the wind. That allows the seeds to be widely distributed. Each cattail ramet can produce at least one inflorescence (flowering spike), which might produce a quarter million seeds. If every square meter has at least 4 cattail inflorescences, it could release a million seeds every year, and even a small marsh (~1,000 m<sup>2</sup>) could release billions of seeds every year. So, if there's a freshwater pool or mudflat downwind, cattail seeds will eventually appear. With a few weeks of wet conditions, the seeds can germinate and establish new ramets, most of which would produce inflorescences at age 2 years.

• Cattails become tall by growing light-weight leaves that are full of air spaces (this is a cheap construction tactic). Comparing a cattail ramet to an oak seedling is like comparing the Eiffel Tower to the Empire State Building—the cattails take a lot less raw material and less energy to grow than an oak seedling. Many species of cattail are highly productive.

• Hybrid cattails can tolerate a wider range of conditions, such as water depth, than either parent. The most well-known case is the widespread hybrid, *Typha x glauca*, which has a native parent, broad-leaf cattail (*Typha latifolia*), and an exotic parent, narrow-leaved cattail (*Typha angustifolia*), which arrived on the East Coast around 1860. Dr. Sue Galatowitsch et al. (1999) published a map that shows how the hybrid (*T. x glauca*) has spread across the US.

• In recent research by Dr. Dan Larkin and collaborators (2012), cattails can take up N from the litter of other neighboring species better than the neighbors can take up N from cattail litter. And cattails can retain (hang onto) that N longer.

Theses are positive feedbacks (as in my simple model on right) because the more N you get, the more able you are to get more N. Cattails shade out other species by being tall and accumulating litter that does not decompose rapidly.



• Cattails were dubbed monotype dominants by Christin Frieswyk in her studies of Lake Michigan marshes. She found that invasive cattails are not friendly to other plant species (Frieswyk et al. 2008)!

## Why did cattails invade the San Diego River salt marsh?

First, let's see what dominated the San Diego river salt marsh before cattails invaded. In 1976,

Bruce McIntyre documented that perennial pickleweed was the sole dominant in the most downstream portion of the marsh.

The pickleweed marsh was not very attractive, because it had just one dominant and it was confined to a flood control channel with riprap on both sides. However, the fact that it was highly disturbed and not diverse made it suitable for Bruce to develop an MS thesis project that involved destructive trampling on the 1976 dominant, perennial pickleweed. There was plenty of room to set up plots for different amounts and frequency and timing of trampling. Of course, the more Bruce trampled, the less pickleweed could grow. The barest areas recovered in 7 months, once trampling ceased (McIntyre 1977). So pickleweed was still dominant in late 1979.

In 1980, floodwaters filled the channel, just as it was designed for. The pickleweed monotype disappeared, and soil salinity was much lower. Either salts washed out (leached) or fresh sediment from upstream was dumped on top of the salt marsh, smothering the pre-flood vegetation. What a change! In January 1980, I went on sabbatical to the United Kingdom. When I returned in June. The former salt marsh was a new cattail marsh. How did such a conversion occur in less than 6 months?



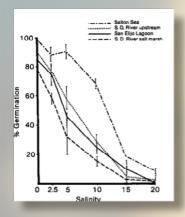
Discovering salt marsh secrets (Zedler 2015)

**Pam Beare** wanted to find out the cause  $\rightarrow$  effect for the shift in composition, and her MS thesis focused on the question: What keeps cattails out of saline marshes? Pam compared seed germination, seedling growth, and adult plant growth of the local native cattail, called southern cattail (*Typha domingensis*). Her experiments involved growing each age group in increasing amounts of salt.

First, Pam studied the seeds, which she collected from the San Diego R. (both downstream and upstream, away from the sea salt), at San Elijo Lagoon, and at the Salton Sea, predicting that the Salton Sea seeds would be the most salt tolerant, because they were coming from the most saline marsh.

Pam invented a clever system for testing germination in fresh and brackish water. She used small squares of styrofoam meat trays to fit in a petri dish, then added water of the desired salinity, put a circular filter paper over the top, so the edges would wick up the solution (see drawing in chapter four). Using a moist toothpick, she touched a seed and transferred it to the filter paper—5 rows @ 5 seeds = 25 seeds per petri dish.

Try testing the effects of salt solutions at home (you can use a jar lid and wrap it in a Ziploc bag if you don't have petri dishes). You can test solutions up to  $\sim 3 \%$  NaCl (3.4-3.5 % mimics seawater salinity) to see effects. Dip the toothpick into the test water (wetting the tip of allows a single seed to stick long enough to make the transfer). With 25 seeds in place, close the lid and use a strip of parafilm (or tape) to wrap and seal the dish from water-loss, which would change the salinity of the solution. Now, watch the seeds for 4-6 weeks, so they have a change to germinate or to show a delayed-germination effect. Salt can either damage the seed or induce dormancy. Six weeks is a suitable observation period. After the test period, you can pour off the saline water and replace with freshwater. If the seeds germinate in freshwater, then salt delayed germination.



Pam found that cattail seeds did not germinate readily in salty water, although, as predicted, the Salton Sea population was more salt-tolerant than other seeds that she tested. Only the Salton Sea population could germinate at 20 ppt salt, and then germination occurred very slowly, with less than 10 of 100 germinating. In freshwater, 100% of seeds germinated very quickly. The upstream and downstream San Diego River populations had similar, but not identical, germination rates.

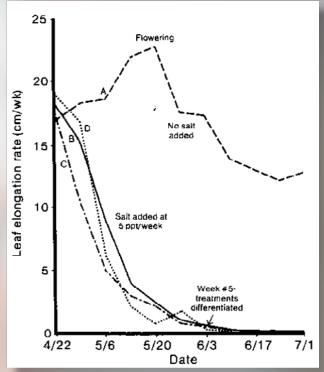
Second, Pam tested the salt tolerance of cattail seedlings: She grew seedlings in various salt treatments. Would you predict that seedlings would tolerate more or less salt than seeds? The seedlings were more tolerant initially, but seedlings exposed to salt at 8 weeks of age were less tolerant that seedlings exposed to salt at 1 week. It seemed that the seedlings could acclimate (get used to some salt) if introduced to it at an early age.

That might sound familiar in the context of food preferences: the first time you ate spicy food, you probably couldn't tolerate as much chili sauce as those who grew up with the stuff.

Third, Pam studied the salt tolerance of adult plants (ones with rhizomes). She dug up ramets (a piece of a plant that can grow independently, in this case, leaves with an attached underground stem) to test for greater salt tolerance than for seedlings. She collected ramets <20 cm tall from the San Diego River flood control channel and planted them outdoors at SDSU in watertight containers. She gradually added Instant Ocean® (artificial seawater) to raise the culture salinity by 5 ppt per week to reach 45 ppt (4.5%) for the maximum saline treatment. After 9 weeks, she compared growth by measuring the length of the tallest leaf for each ramet.

As soon as salt was added, leaf growth (elongation rate) slowed from 18 cm/week to zero over a 5-week period. Data are for leaves of ramets grown without salt (A) and with salt increasing by 5 ppt each week (B, C and D). The salt treatments were the same until week 5; then B remained at 25 ppt while C continued to 35 ppt and D to 45 ppt.

Do you think these plants were dead or could they revive if given freshwater after 9 months of salt treatment? Pam wanted to know, so she watered them with freshwater for 3 more months, and 5% of the ones she subjected to 45 ppt salt were still alive and able to start growing again. What a tough plant! (Beare and Zedler 1987).



**CONCLUSION**. Cattails can expand into salt marshes given a "low salinity window." The seeds are the limiting stage; if they can germinate while conditions are fresh, their seedlings can tolerate some salt, and if the seedlings can grow to be adults, they can tolerate even more salt. And even if cattails in hypersaline soils appear to be dead, some might revive with another pulse of freshwater.

These results explained the 1980 invasion of cattails into the San Diego River salt marsh, the reduced area of cattails in 1981 and 1982. After Pam finished her research, the 1983 flood, with rainfall and freshwater flows continuing in March and April, validated her findings—cattails expanded once again, influenced by freshwater!



Cattail seedlings colonizing bare mud in WI.

## Why do algae "bloom" after floods?

Algae don't have flowers, so it seems strange to call a profusion (large quantity) of algae an "algal bloom." I'm not sure who was first to use this term, but it has become a household word. People who swim in nutrient-rich (eutrophic) lakes know that algal blooms are slimy and make the water taste bad. People who fish in eutrophic lakes know that some algal blooms are so thick that they shade the deeper water and cause fish kills. People in Toledo, Ohio, know that some algal blooms are toxic and can poison their drinking water. Below is an aerial photo of an algal bloom in Lake Erie (by Peter Esseck, date not given). • Wastewater is just that—water full of wastes. That can mean treated sewage or street runoff or both. Most modern cities have separate pipes to carry treated sewage and street runoff downstream, but it was not always the case. Before these sources of wastewater were separated, the combined-water pipes filled up with street runoff, mixed with sewage and overflowed where there were manholes or channels. These were major sewage spills, so cities invested in separate systems one to convey stormwater directly to downstream outlets and another to carry sewage to wastewater treatment plants.

• Treatment means allowing the "solids" to settle out and allowing some of the organic matter to be "digested." The resulting "treated" wastewater still has



nutrients and dissolved organic matter, and the water is still fresh, not saline. It is also has chlorine added where wastewater treatment plants use chlorination to kill harmful micro-organisms. Where cities are not too close together along a river, the treated wastewater discharged by an upstream city can undergo enough

Yuck! But please don't blame the algae. They are just the messengers. They are telling us that the water is too eutrophic. Can you guess who adds all those nutrients to the water? We do. Each one of us has some impact on water quality. For example, we all produce sewage. Yuck again. Perhaps you think that treated sewage has no effect on lakes, because it is "treated." But while treatment removes pathogens (disease-causing organisms), it does not remove all the nutrients that stimulate algal blooms. So, here's a brief summary about wastewater and how it is "treated"....or not. further treatment by Nature before the next city downstream draws it into a filtration plant to make it safe for drinking.

• At the time of our earliest studies of Tijuana Estuary, there was no international wastewater treatment plant at the US-Mexico border. Thus, untreated (raw) sewage flowed from Mexico down the Tijuana River, into the US, and into Tijuana Estuary. During all the low-flow years the health risk wasn't horrible, because much of the water soaked into the dry river bottom. But with major floods, beginning in 1978, it was obvious that something had to be done to protect people, the beaches in Imperial Beach, and the rare species in the estuary.

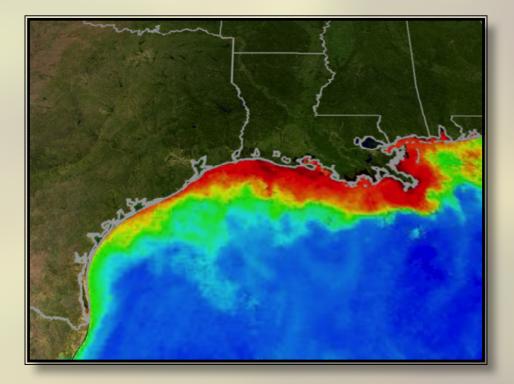
• Decision-makers decided to build treatment plant just north of the border, treat the wastewater from Mexico, and discharge the treated wastewater into a buried pipe that goes under the estuary and discharges deep and far offshore. This system handles 25 mgd (million gallons per day). What if the Tijuana River is flooding? If flood flows exceed 25 mgd, the river still carries untreated wastewater to the estuary and onto the beaches.

Discovering salt marsh secrets (Zedler 2015)

If you live near the ocean and your treated sewage flows into the ocean's deep water far offshore, you might cause fewer algal blooms than I do. I work in Madison, a city that treats its sewage and discharges it to a river south of the wells that supply our drinking water. Our nutrients don't harm us, but they do flow downstream where they can harm other cities along their route to the Gulf of Mexico.

The "dead zone" at the mouth of the Mississippi River is caused by algal blooms that are caused by the nutrients from upstream wastewater treatment plants, agricultural fields, urban street runoff, and many other sources. And because the Mississippi River collects water from about 40% of the land area of the lower 48 states, a lot of "yuck" flows out the mouth—enough to create a dead zone the size of some eastern states, as big or bigger than New Jersey (size depends on the magnitude of the year's algal bloom).

Here is a NASA satellite image during summer. Red indicates the highest turbidity, which refers to particles in the water, especially the density of algae and sediment. The phytoplankton (free-floating algae) extend from the Mississippi River mouth east to Alabama and west along Texas.



When the algal cells die, they sink, and the decomposing bacteria use up the water's oxygen, creating a deadly, hypoxic (low-oxygen) environment for marine organisms. Shrimp and other coastal fisheries are greatly harmed.

#### When do southern CA estuaries risk algal blooms?

If freshwater flows into the estuary as a brief pulse that coincides with an outflowing tide, would the river have much effect on the salt marsh? No. The freshwater would flow straight toward the ocean.

How about when the river flows for several days, especially during a spring tide series? Yes, the river would affect the salt marsh then. As the freshwater moves downstream, inflowing tides each day would act like a dam—a wall of seawater that would block the outflow, forcing the freshwater to spread into estuarine creeks and channels. Because freshwater tends to float over seawater, the river waters would move a layer of low-salinity water into tidal creeks and pools.

In flood years, we noticed "algal blooms" in the tidal ponds at the far northeast end of Tijuana Estuary. The waters were probably not well mixed or diluted by low-nutrient tidal water. A hypothesis was that nutrients flowed into the estuary with the freshwater and were detained in shallow pools, where algae could grow rapidly and accumulate.

Nutrients move from the land toward the ocean in river water. This next aerial photo of the Tijuana River Valley shows a lot of the land just upstream from the estuary in cultivation. It erodes easily. Other areas are urban and have increased runoff due to hardscapes (roofs, sidewalks, streets). Those areas discharge nutrients year round when people irrigate and fertilize their lawns.



Discovering salt marsh secrets (Zedler 2015)

Visit the salt marsh after a rainfall event, and look the high-tide line. You can tell where some of the runoff comes from when large pieces of telltale (identifiable) debris flow and accumulate in the estuary. Many stakes from tomato fields washed downstream one year.



Now, with many large cities and so many people living near our coastal wetlands, there is additional urban runoff with sediment, debris, nutrients and other contaminants. Here's an example near the Tijuana Estuary Visitor Center.

## How do changes in salinity & nutrients affect estuary water?

Both plants and animals are affected. The plants I'm talking about are the tiny planktonic (free-floating) algae that you can see with a microscope (microalgae), and the algae that are larger, called macroalgae. Both thrive in water, not on land. What makes them "algae" and not land plants? They are very primitive plants that don't have leaves, stems or roots. Instead, they are single cells or form colonies of cells or filaments with branches, or multi-cellular blades, like the seaweed "nori" that you might enjoy as a sushi wrapper.



The first key is water—algae float about in water. They can live on land if their spores find a moist or wet refuge and secrete some "glue" (mucus that soaks up moisture) to prevent desiccation (drying). It's much easier for algae to absorb nutrients from water than for land plants to obtain nutrients from soil. All the algal parts can take up nutrients, so algae don't need a system of tubes (vascular tissue) to move water from one part of the plant to another. And algae don't need leaves to absorb light. The entire alga, like this *Chaetomorpa*, can absorb light! Of course, algae that have leaf-like blades and bladders for flotation (like the giant kelp) can grow in deeper water—but they need a holdfast to anchor their stipes (stem-like structure). Most estuary algae don't need large holdfasts, but some have tiny holdfasts that allow them to attach to a shallow-water substrate.

When the alga becomes large enough for water currents to break it loose, the algal filaments or blades float to the surface and absorb more light (but risk floating away). Once the algae are "set free," they can waft onto the plant canopy at high tide and get "hung out to dry" during low tide. That's not a good thing from the algal point of view. Sometimes, the salt marsh looks like it has tissue paper strewn over the canopy. A closer look might reveal that they are bits of a sunbleached algal mat.



Algae don't need roots to absorb water and nutrients. A few modified cells that secrete a sticky "glue" can attach to a substrate. On the left is sea lettuce (*Ulva*, now lumped in the genus *Enteromorpha*), which grows a large, thin and very flexible blade that is anchored by a tiny holdfast.

Unlike land plants, algae don't need stiff stems; in fact, water currents and waves would break stiff structures. Under water, it helps to be flexible. *Enteromorpha* species are estuarine examples of algae that are free-floating most of the time.

(forums.saltwaterfish.com/)

#### A superteam tackles estuarine algae

Two very talented researchers learned how nutrient-rich inflows affect estuarine algae. Read on....

Regina Rudnicki focused her graduate research on the macroalgae. Peggy Fong developed her career studying both micro- and macroalgae. Luckily, she didn't mind mud  $\rightarrow$ .

We hypothesized that the phytoplankton would soak up nutrients and reproduce rapidly and that macroalgae would not be far behind. In contrast, vascular plants in the tidal marsh would respond slowly.

Peggy and Regina decided to "divide and conquer," Peggy to work on the phytoplankton and Regina to tackle the macroalgae that are loosely-attached and often break loose and float where the water moves them.



In 1984, Tijuana Estuary had extensive algal blooms, so we expected to find them again in 1985. We didn't, despite considerable effort. First, Regina and Peggy needed a boat so they wouldn't have to walk in mud, disturb the algae of interest, get stuck...or spend time trying to get unstuck.

First, the team tried using a canoe, but it was too tippy for leaning over to collect water samples and too long to navigate sharp bends in the tidal creeks. One day, the canoe slipped and attacked Peggy, leaving a scar on her chin after she and Regina tried to remove it from the car roof. Peggy said it was "not my best decision" trying to catch it. Because sewage was entering Tijuana Estuary at the time, antibiotics were needed to keep Peggy's wound from becoming infected.



Peggy with plastic raft. Photo: Rudnicki

Next the team tried a "dime-store" raft that was inflatable, but it popped one day when they were paddling in one of the tidal creeks.

To the rescue: Chris Donohoe, who seemed to have a motive for helping Regina, built a small boat, tailor-made for tidal-creek sampling. Peggy described it as "flat bottomed, short and fat... Worked like a charm."

Peggy, Regina, Chris and other graduate students took my Aquatic Ecology class at the same time. It led to lasting friendships and more! Regina's surname is now Donohoe.

Although the estuary water looked like "pea soup" in 1984 (during the 8-mo. nontidal period), restoring tidal flushing in 1985 reduced blooms of phytoplankton and macroaalgae. How come? There would have been many confounding factors from year to year, but hte most important was probably tidal export—the dilution of algae by the

inflowing tide and the removal of algae by

the outflowing tide.



Regina in Navicula. Photo: C. Donohoe.

Below is an algal bloom at Los Peñasquitos Lagoon when the ocean mouth was closed. Water trapped inside the estuary develop an algal bloom when the tides couldn't flush out the algae, when the litter in the marsh decomposed and released nutrients, when inflowing nutrients couldn't flow out into the ocean, and probably many other reasons.



Peggy and Regina simultaneously monitored planktonic algae and epibenthic macroalgae in Tijuana Estuary during 1985.

#### MEASURING MICROALGAE:

• To monitor: Peggy and Regina used a 1-m-long glass tube (2 cm dia) to obtain a complete water column profile—a technique that our colleague Stuart Hurlbert had developed for sampling shallow water. You hold it vertically and gently push it into the water, then cork it at the top, remove and pour the trapped water into a sample bottle.

• Back in the lab: They filtered the water through a membrane filter with pore sizes small enough to collect all the photosynthetic phytoplankton. Then they dissolved the filter in acetone to release the chlorophyll. The acetone becomes bright green when the algae are dense. Next, they poured the fluid into a special 1-cm square tube (vial) and placed the vial in a spectrophotometer to measure how much light the fluid absorbs. "Spectro" refers to a broad range; "photo" specifies that this is about light waves; and "meter" just means an instrument that measures something. The spectrophotometer focuses light beams of the specific wave lengths that chlorophyll (green pigment in plants that absorbs light) absorbs; the more chlorophyll in the fluid, the more light the fluid absorbs. The measurement is the reduction of light (light transmitted/light provided). Cool!

• Well, it's a little more complicated that that, because there are 3 kinds of chlorophyll and you need multiple measures of light attenuation from each wavelength....but the general idea is that the job of chlorophyll is to trap light for photosynthesis, so it makes sense that the amount of chlorophyll can be determined by how much light it traps in a spectrophotometer.

#### **MEASURING MACROALGAE:** How would you measure the growth of macroalgae?

Unlike microalgae, the strands of *Enteromorpha* are easy to remove, measure, and replace so they can continue growth. At first, Regina removed them and placed them in a graduated cylinder to see how much water they displaced. Later she used a "lettuce spinner" (appropriate, since green macroalgae are often called sea lettuce). Spinning removed excess water so she could use a spring scale to weigh them in the field. Probably every researcher develops methods that improve efficiency along the way. The more inventive you are, the more you'll contribute to science!



#### Testing nutrients and salinity

Because we could not rely on a field study to provide publishable data within a 2-year MS degree program, we added experimental approaches. By controlling salinity and nutrients, they could test for cause→effect. The team set up field experiments in 15-liter plastic "microcosms"—a more professional term than "buckets" or "tubs." They placed replicate microcosms in a rack anchored in a tidal channel, so that the temperature and light conditions would mimic Nature. They applied nutrient and salinity treatments, then seeded the water with a sample containing both macroalgae and estuary water. Thus began a series of microcosm studies that led to larger mesocosm studies.

**EXPERIMENTING OUTDOORS**: While it's an advantage to grow algae outdoors with realistic variations in light and temperature, it's a real disadvantage when you can't know exactly what all the environmental conditions are. As a result, outdoor experiments are difficult to repeat. Another disadvantage in this case involved birds (see below). Peggy and Regina could not test the effects of all the other things—like organic matter, sediment, heavy metals, and pollutants that runoff collects on its way from the watershed to the wetland. Such analyses cost a lot of money and require additional specialists.

Peggy and Regina chose 3 salinity levels, 10, 20, and 33 parts per thousand (1, 2, and 3.3%), the latter of which represented seawater. They created 3 wastewater additions (low, medium and high doses) by adding pellets of Milorganite (dried Milwaukee sewage available commercially as a soil conditioner. Milorganite has a ratio of 3:1 for nitrogen to phosphorus (Rudnicki 1986). If you're concerned that they did not add nitrogen (N) and phosphorus (P) independently, stay tuned for Peggy's doctoral dissertation research. The experimentation with Milorganite led to further analyses of the effects of N and P alone and N + P in various ratios.

Testing 3 salinities each with 3 levels of Milorganite led to 9 treatments x 3 replicates = 27 microcosms. But what about the weather? To find out if there were seasonal differences, they repeated the experiment in winter, spring and summer--each time for three weeks. They set up the first experiment in March 1985. But uninvited birds seemed to think the experimental containers were installed for their benefit. To keep them from adding their own effluent to the microcosms, Peggy and Regina added spikes to the rack to discourage bird-perching during the May and September repetitions of the experiment. There are always surprises with outdoor experimentation, so a good researcher pays attention and makes changes as needed, making sure that the desired treatments are actually testing the hypothesis.

ALGAL DYNAMICS: Even though algae were less abundant than in 1984, the team could still measure phytoplankton chlorophyll and macroalgal biomass. We were surprised by the lack of algal blooms in 1985, but we explained the observations as follows: After tidal flushing was restored, the tidal seawater diluted the warm, nutrient-rich estuary water. Seawater reduced algal growth. Both micro- and macroalgae were more abundant in March, April and May than in summer or winter. Because the least tidal flushing occurs in springtime, more cells could grow before being diluted by tidal water.

The most abundant phytoplankton were diatoms (single-cell algae with a silicon shell), unidentifiable tiny unicells (one-celled algae), and dinoflagellates (unicells that have 2 flagelli, hence "dino", or moveable tails, that allow them to swim).

The macroalgae began settling and growing on the creek bottom and edges in February, then expanding and breaking loose to float in March. That was when they reached peak "cover" (intercept along transects measured while paddling from channel to channel in the boat, Navicula). Floating mats disappeared again by October.

In the microcosms, low salinity and high nutrients stimulated macroalgal growth, but with interactions (see below) between the two variables compared and due to season and due to the presence of phytoplankton. The micro- and macroalgae had peak responses at different times—macroalgae in winter and phytoplankton in spring, but more complicated responses to salinity. When nutrients were added at a high level, phytoplankton peaked before macroalgae; when low levels of Milorganite were added, the opposite pattern occurred. Is it any wonder that in nature, temporal and spatial patterns are hard to characterize? Under natural conditions, there are also herbivores and carnivores to contend with, as well as tidal dilution and mixing. What's an interaction between environmental factors?

• In Peggy and Regina's experiment, the effect of nutrients depended on salinity and season; in other words, the algae responded to nutrients with different salinity treatments when they set up the microcosms in winter vs. spring. The cool thing about their "two-factor experiment," with salinity and Milorganite varied alone and together, is that a statistical analysis can identify that interaction as "significant" or not.

• Significant interaction meant a strong effect of salinity on the response of algae to nutrients. Somehow, the phytoplankton interacted with the macroalgae, since both were present in all microcosms—just like in the creeks and channels. The team's test of nutrient and salinity on the combined algae components was realistic. If they had grown phytoplankton alone, macroalgae alone, and the two in combination—that would have required many more treatments: 3 salinities x 3 nutrient levels x 3 algal treatments = 27 treatments. Then multiply by 3 or more replicates of each treatment. That's a lot of containers to set up, sample, and analyze.

WHY DID THE SEASON MATTER? Why were there seasonal shifts in the abundance of three groups of algae (green macroalgae, cyanobacterial mats, and phytoplankton) that co-occur in shallow coastal lagoons of southern California? Peggy decided to test their responses to temperature, irradiance (sunlight) and daylength.

She set up 2-liter aquaria and maintained them in environmental chambers. She added the three groups of algae and followed their responses. The biomass of phytoplankton and mats was highest at high temperature (25 °C). In contrast, macroalgae had maximum biomass at 18 and 21 °C. Reduced light and short days favored phytoplankton and attached macroalgae. High light and longer days favored floating macroalgae and bluegreen mats. Our mixed-algal-community experiments helped to explain the seasonal sequence in southern California coastal lagoons:

- Attached macroalgae dominate in early spring.
- Floating macroalgae dominate in summer.
- Bluegreen (cyanobacterial) mats dominate
- in late summer and early fall.
- Phytoplankton dominate in late fall (Fong and Zedler 1993).

Regina: "I thought it was cool that the abundance of minus tides during the daylight hours in Jan/Feb could have provided more light to the macroalgae, also promoting growth. In the salt marsh channels, the estuary had "pea soup" in 1984, when it was nontidal for 8 months, and relatively clear water in 1985. Restoring tidal flushing reduced phytoplankton abundance. In the tidal creeks, the ratio of N:P was variable over time and among tidal creeks. When N was much more abundant than P, N was not limiting. At such times, algal growth was most likely limited by P. One of the 5 tidal creeks monitored had an especially high ratio of N:P during summer. The actual data are really complicated. Still, Peggy made sense out of it so that decision-makers could use the findings:

• In microcosms, Peggy found that Milorganite stimulated phytoplankton in all seasons, but not at the lowest salinity. Milorganite increased phytoplankton exponentially! That means they grew by orders of magnitude, from 200,000 to 2 million cells within a week!

• Regina found that Milorganite increased macroalgae at all salinity levels but not in all seasons. In winter and spring, macroalgae grew exponentially but not in September, when the estuary lacked macroalgae.

• Contrary to expectations, low salinity increased phytoplankton abundance! It was the tiny unicells that surprised Peggy. They were abundant with high addition of Milorganite at seawater salinity (33 ppt); while diatoms were most abundant with high addition of Milorganite under brackish salinity (10 ppt). In between (20 ppt), composition was mixed. Nice results.

- While Milorganite had its biggest effect on abundance, salinity had its greatest effect on composition: Milorganite  $\rightarrow$  abundant algae; salinity  $\rightarrow$  type of algae
- Not surprising, the presence of macroalgae interfered with phytoplankton responses, perhaps by luxury uptake (absorbing more nutrients than required at the time).

In the estuary, releasing treated wastewater would increase algal growth and turn a seasonal stress (low salinity) into a persistent stress. Phytoplankton composition in summer would shift toward diatoms instead of small unicells.



Sediments also contribute nutrients and act as sinks..... it is hard to know how much effect river inflows or wastewater discharges will have, but the monitoring and experimentation leads to useful predictions.

In 1997, the excavated Tidal Linkage had a macroalgal bloom (photo on left). The channel excavation work extended west into salt marsh soil and east into the sediment of the historical sewage lagoon. One hypothesis is that excavating nutrient-rich sediments mobilized nitrogen and phosphorus.

The green macroalgae formed a mat that floated during high tide. When the tide receded, the mat dropped onto the marsh plain, smothering some newly planted seedlings in our salt marsh diversity experiment. A greater threat than being smothered by algae, however, were the coots (*Fulica americana*) that arrived to feed on the algae. Their trampling damaged seedlings, which we had to replace, once we figured out how to deter coot trampling. Amazingly, a short chickenwire fence was effective. While the coots had flown onto the open marsh plain, they avoided our fenced area.



### Which nutrients trigger algal growth?

In Nature, N is sometimes limiting and P is sometimes limiting. If you add N by itself and get a large growth response, and if adding P in other containers gives you a smaller or no response, then N was more limiting.

If you add both N and P, you will probably see the largest response. Neither the chemistry of nutrient availability nor the physiology of plant uptake and growth is simple. There's a tendency for N to be more limiting in coastal waters, but this idea is being challenged. Sometimes, broad generalizations are based on too few studies.

When Peggy decided to continue graduate school toward a PhD, she wanted to test the basic ideas about N and P in runoff. She asked: Does one or the other or both might stimulate an algal "bloom" and/or some specific ratio of N:P have a stimulating effect?

• First, we designed an experiment that would compare amounts of N, amounts of P and ratios of N:P. Why ratios?--Because marine organisms were widely known to accumulate N and P in ratios of dissolved inorganic N and P that were ~16:1. At the time, no one had tested the concept, either in the lab or in Nature. Inquiring minds wanted to know!

• We found a source of affordable plastic barrels, bought 60 of them, then cut each in half to make a total of 120 mesocosms, each able to hold 100 liters. We placed them outdoors at PERL, near the US-Mexico border. We trucked in seawater from Scripps Pier, and had freshwater piped to the site. We could then vary salinity and replace evaporated water. With the mesocosms filled with water, the next step was "seeding" them with estuary water, then "treating" them with various amounts of the key nutrients for algal growth, N and P.

• Mesocosms were inoculated in summer with three algal groups and subjected to four levels of nutrient enrichment in five N: P ratios (20 treatments, n = 4). A subset of 12 treatments was repeated in spring. Algal groups were green macrophytes, phytoplankton, and benthic mats of cyanobacteria with associated epiphytes.

For the twenty N and P treatments, N-limitation was more common than P-limitation in summer (14 of 20 treatments). Differential limitation was not as clear in spring. P supply appeared to be more important to the bluegreen mats, especially in spring.

N directly controlled macroalgal biomass, and macroalgae controlled the biomass of mats and phytoplankton. Only when there was N left over from macroalgal use, could other algae thrive. Thus, Fong et al. (1993) hypothesized that macroalgae efficiently stored some of the N and outcompeted the other groups. The differences among algal groups and the temporal variation in N and P limitation helped explain why N and P are both implicated in nutrient limitation.

**COMPETITION**. Did the estuarine phytoplankton and algal mats compete for nutrients? The algal mats combined floating and attached green macroalgae and attached cyanobacteria in this study. Time for another experiment, with nutrient additions with and without algal mats.

At high nutrient loading rates, the phytoplankton growth was reduced by a factor of 10 in the presence of the algal mats. Without the algal mats the phytoplankton was very abundant and dominated by small flagellates, while in the presence of the algal mats the phytoplankton assemblage was sparse. Diatoms, flagellates, and unicellular bluegreens were common. The competition hierarchy was cyanobacterial mats >> attached green macroalgae > floating green macroalgae > phytoplankton. When nutrient supply rate was low, algal mats shifted the phytoplankton from flagellates to bluegreen algae, but did not affect total phytoplankton biomass. We concluded that the attached forms of macroalgae as well as the cyanobacterial mats were better competitors for high levels of nutrients than the phytoplankton. Resource competition could explain negative correlations between phytoplankton and macroalgae in shallow nutrient-enriched estuaries (Fong et al. 1993b).

**N STORAGE?** Recall the earlier suggestion that macroalgae might take up extra nitrogen and store it for later use? Peggy and Regina set up another experiment to assess the relationship between nutrient concentration in the tissue of *Enteromorpha* and the algal's history of exposure to nutrients (water-column concentration or supply rate). Experimental units were outdoor mesoocosms containing mixed assemblages of algae representative of communities commonly found in coastal lagoons of southern California, USA. We determined the relationship between nutrient supply rates (N and P, as well as N:P ratio), water-column nutrient concentrations, and macroalgal tissue nutrient concentration in the mesocosms with macroalgae, phytoplankton, and bluegreen mats, all of which had been treated with 13 combinations of N and P addition.

What was the outcome? Tissue P, P supply rate and water-column P were all strongly correlated with nutrient history! The same relationships for N weren't quite as strong. The relationship between tissue nutrients and either measure of nutrient history (water-column concentration or supply rate) was most useful when the nutrient was not limiting (Fong et al.1993c).

#### Why are N and P the key nutrients?

It has to do with the plants' needs and the availability of each nutrient in Nature. If a plant has a high demand for a nutrient that is very rare, that nutrient would be limiting to its growth. On the other hand, if a plant has little demand for something very abundant, it would lose out in competition with another species that could take advantage of the abundant nutrient. So, Nature makes sense:

<sup>•</sup> N is abundant in Nature because the gas form,  $N_2$ , makes up almost 80% of the air and because some bluegreen algae (cyanobacteria) have evolved the ability to convert N gas into amino acids (N-containing building blocks of proteins). In water bodies, these bluegreen algae "fix" more N when the concentration is low and they tend to shut down production when the N concentration is high—it's an energy-requiring process, so they don't bother making a lot when a lot is already available. They can use their energy for reproduction instead. Waste not, want not. So, when N becomes limiting to plants that can't fix N, they don't grow very well.

• P is rare in Nature, because it has no gas form (greatly restricting its mobility) and it has to be eroded from rocks or recycled from organisms that have already taken up the P and died, making P available for decomposers to release again. You can find a lot of P at the bottoms of lakes, because it sticks to clay particles that sink in the water column. But what good is a layer of P-rich clay to an algal cell near the water surface? It might as well be locked in a bank. In fact, the next layer of sediment that flows into the water body will do just that—lock it into the sediment P bank. Of course, a major storm can erode the bottom and stir the sediment up into the water column, liberating the trapped P and giving it another chance to feed the algae (or vascular plants) in the estuary. But that happens rarely for most of the P that falls to the sediment. The point is that P is relatively rare both in nature and in organisms, but still very important as our "energy currency." Specifically, adenosine triphosphate (ATP) is the energy currency of all living organisms. ATP receives and gives up energy in the process of making and decomposing organic molecules. So when P becomes limiting to plants, they don't grow very well.

Later Fong et al. (1996) suggested that *Ulva* (now a species of *Enteromorpha*) might leak enough N to facilitate growth of *Enteromorpha*, in her research to explain the order of algal dominance in estuaries and lagoons of southern California. Peggy thought that differential tolerance to low salinity and competition for N would explain the seasonal pattern, but it was more complicated than a simple model, salinity  $\rightarrow$  superior competitor. Experimentation with lowered salinity was unfavorable to both algae, except when N was added. With ample N, half-seawater (15 ppt = 1.5%) favored *Enteromorpha* while seawater (~35 ppt; 3.5%) favored *Ulva*. When grown together, *Enteromorpha* outgrew *Ulva*. But *Ulva* actually facilitated the growth of *Enteromorpha* when N was in short supply. Fascinating! Peggy suggests that dissolved organic nitrogen could leak out of *Ulva* when it becomes starved of N. *Enteromorpha* would bloom shortly after a rain event, but ample N and seawater salinity would shift dominance to *Ulva*. Later, at the end of the rainy season, *Enteromorpha* would outcompete *Ulva* when N becomes scarce. Just who dominates when and where is as complicated among macroalgae as it is among the vascular plants. What fun unraveling all the interactions!

#### How can we reduce nutrient inflows?

Runoff can be managed in many ways, by trapping it in retention ponds, by diverting it around nature reserves, and by using it as a resource. Can you imagine how? Read on...

Some decades ago, local managers at Tijuana Estuary noticed that people were discharging more and more water into street gutters at the corner of Caspian Way and 3<sup>rd</sup> Street in Imperial Beach, just west of the Visitor Center. With more housing, there were more upstream residents, who collectively washed more cars and watered more lawns and produced more runoff that flowed downstream into the salt marsh. The managers engaged an engineer to "solve" the problem. The resulting design involved installing a system of trestles to hold a large pipe that would transfer the runoff directly into the salt marsh. I saw the drawing and swallowed hard. It was more than ugly; it was a major threat to the marsh vegetation and the estuary's water quality. I suggested instead that street runoff could be diverted around the salt marsh by creating a small creekbed along the edge of the upland area. It wouldn't damage any native habitat, because the substrate was filled material from historical dumping. I predicted that much of the water would soak into the soil before it reached the salt marsh.

I was delighted when the idea became reality and amazed at how well it worked! Not only did the marsh gain protection, the creekbed facilitated the growth of riparian (streamside) vegetation. The photo below, taken in 2014, shows a band of willow trees that hide the artificial creek, as well as a variety of birds. The adjacent path allows the public to appreciate the birds and view the salt marsh. Any water that the willows don't take up, trickles along the artificial creek bed, where most of it soaks into the ground or evaporates. In contrast, coastal scrub vegetation on the left tolerates dry conditions.

This small project demonstrates how a city's waste (urban runoff) was turned into a resource.



The riparian system is self-sustaining, now that the trees have deep roots and can thrive between inflows. Contrast that with the alternative engineering plan to convey the runoff to the salt marsh in a large pipe. The pipe and the trestles would have required continual maintenance, as does all infrastructure (basic things built to support city life, including systems to supply water supply, treat sewage and manage runoff).

## The pulsed-discharge idea for managing runoff

Most problems with excess freshwater flows to estuaries are much larger than the trickle that flows intermittently (from time to time) from a small urban neighborhood. Wherever people concentrate, there is a lot of wastewater—some from street runoff during storms (stormwater) and some from sewage that is discharged downstream after being treated (cleaned up) at a wastewater facility (treatment plant—meaning a facility, not an organism). Large volumes of wastewater cause multiple problems for estuaries—the water dilutes the salinity and adds nutrients and other contaminants (materials that can be damaging or toxic). Treatment plants reduce the contaminants but there's still a lot of water flowing toward the estuaries.

In southern CA, most of the water people use has its origin far upstream, even from other watersheds. Water from northern CA arrives via a system of pumps and aqueducts that carry it over the San Bernadino Mountains. As often said about CA, all the water is in northern CA, while all the people are in southern CA. Is it easier to move the water than the people? Is it wiser?

The sophisticated (highly engineered) water supply system makes it possible for millions of people to live in southern CA. Water disposal, by contrast, has no comparable system of management. Excess water just flows downstream and ends up in the ocean. Negative effects on the salt marshes and estuaries in between the ocean and the treatment plant pose major challenges, because there is no simple alternative to "letting it flow."

**FRESHWATER TIDAL WETLANDS**. Along the Atlantic Coast, there are many freshwater marshes that occur just upstream from the salt marshes and estuaries. These freshwater marshes are supported by rivers that convey natural freshwater runoff (from rainfall and large watersheds) toward the coast. Where the freshwater of the river meets the seawater in tidal channels. Every time the tide rises (usually a diurnal tide with two similar high waters per day), the freshwater is blocked from flowing downstream, so it rises and falls with the tide, even though the water is fresh, with some brackish conditions at the fresh-saline boundary. The adjacent mashes are called freshwater tidal wetlands.

I was inspired by descriptions of such wetlands. Various studies discussed their high productivity, their ability to remove nutrients from river water, and their high diversity of native plants and animals. Freshwater tidal marshes have all the benefits of inflowing and outflowing water without the stress of salt. No wonder they are among the world's most productive and diverse ecosystems. A recent book by Barendregt et al. (2009) is good reading; each chapter is an expert review about the many aspects of tidal freshwater wetlands.



(photo: Virginia Dept. of Env. Quality)

It was a dream that discharging wastewater in pulses to coincide with ebb (outflowing) tides would solve the problem of estuary dilution. Freshwater could be sucked out to sea before it had much time to dilute or contaminate the salt marsh! There might be other benefits, too. The literature says that tidal freshwater wetlands remove contaminants. Nitrogen removal via denitrification is one of their specialties.

**DENITRIFICATION**. For decades, scientists have known that wetlands remove N, and that denitrification is enhanced (improved) by *intermittent* exposure to oxygen--not constant aerobic conditions and not constant anaerobic conditions. Freshwater tidal wetlands have rising and falling water that aerates and waterlogs the soil twice a day, so they should (and do) remove lots of N. A second key to denitrification is an ample supply of nitrate, which is abundant in river waters that collect nitrogen from upstream fields and lawns. Could we mimic the hydroperiod (wet-dry conditions) of a tidal freshwater wetland to increase denitrification?

We hypothesized that alternating aerobic and anaerobic conditions would remove more N and heavy metals than constant water levels. To test that hypothesis, we'd have to control water levels while allowing a wetland to remove nitrogen. Which hydroperiods (durations of high water) should we compare? Alternating flooding and drainage like a tidal freshwater wetland? Would wetlands with twice-daily inundation and drainage remove more nitrogen (and perhaps also phosphorus and maybe even heavy metals) than wetlands with more prolonged hydroperiods?

Drs. René Langis, Rick Gersberg, and I put our heads together, aided by two graduate students:

Max Busnardo and Theresa Sinicrope, who wanted to work on nutrient and heavy metal removal as an example of contaminants. We had support from the California Sea Grant Program, but not enough money to analyze organic contaminants, such as pesticides, grease, and oil. We had a fenced facility near the Tijuana Estuary visitor center; and we had access to running water and a small storage shed.

That was our second Pacific Estuarine Research Lab (PERL2)--closer to civilization after outlaws and vandals had their way with our facility near the US-Mexico Border. More than the facility, we wanted to protect our collaborators. Peggy and Regina, for example, appeared suspicious to the Border Patrol. I admit, they were collecting water samples from mesocosms during the night, so it was a reasonable suspicion. Also it was a clear indicator of nearby crime and criminals. "PERL1" had a tall fence and padlock, but it still wasn't safe.



A plan emerged. We decided to create miniature wetlands in large mesocosms and to test four hydroperiods.

I found a source of oblong fiberglass tubs; they were 1.2 x 1.7 m and 45 cm deep. We added clean river sand and then planted our native brackish marsh plant, California bulrush (*Scirpus californica*, renamed *Schoenoplectus californicus*). We installed 9 ramets per tub, all trimmed to 50 cm height.

You might ask why we used sand instead of clay. Answer: Sand drains more readily and oxygen can penetrate the soil more readily. But there was also a logistical reason--we wanted to collect roots and rhizomes to assess biomass. Anyone who has ever tried to separate roots from fine soils will understand why we avoided clay. Clay particles are so sticky that roots won't let go of them!

#### OUR FOUR HYDROPERIOD TREATMENTS WERE:

- 1. Continuous inflow and outflow, maintaining constant water level
- 2. Every other day: 44 hr inundation, 4 hr discharging
- 3. Once per day: 22 hr inundation, 2 hr discharging
- 4. Twice daily: 11 hr inundation, 1 hr discharge, 11 hr inundation, 1 hr discharge

How many mesocosms did we need? Answer: 4 treatments x 5 replicates = 20



How did we create the four planned treatments? René and Rick figured out how to control water levels. We purchased peristaltic pumps to supply the synthetic "wastewater" and solenoid valves to control the outflows. The synthetic (mixed by us) secondary-treated wastewater included known quantities of cadmium, copper, chromium, nickel, lead, zinc, nitrogen, and phosphorus, as well as organic matter (sodium acetate, C:N = 2:1).

After the plants had grown for about six months, Theresa and Max began comparing concentrations of metals and nutrients in the outflow versus inflow water. They also measured redox within the soil in order to explain changes in soil metal and nitrogen concentrations.



After harvesting the shoots, the students extracted roots with a steel, razor-edged corer (demonstrated by Theresa), then washed the roots over a sieve to separate roots from the sand (Max). Look how tall the rushes grew!



What is redox? It's the reduction-oxidation state, for which there are probes and meters. When aerated, soil has a high redox potential; when anaerobic, redox is very negative. We measured redox to learn if our treatments provided the aerated and anaerobic conditions suitable for removal of N and perhaps heavy metals. NOW FOR THE FUN PART-THE DATA! We harvested the plants and roots and rhizomes and found astounding biomass production. I still remember Rick having difficulty believing the numbers. He was certain we had made errors calculating kilograms per square meter. Natural marshes sometimes produce 3 kg/m<sup>2</sup> aboveground (weight of plant after drying in an oven); our tubs produced 11.6 kg/m<sup>2</sup> aboveground and a total of 17 kg/m<sup>2</sup> for above plus belowground. That equates to about 164,300 pounds (82 tons or ~16 African male elephants!) over an area the size of an NFL football field. Rick was right to be skeptical!

By looking carefully for errors, we did discover that our biomass data were overestimates, because the plants were hanging over the edges of the tubs and harvesting light from an area larger than the tub area (see photos above). So we corrected the aboveground biomass estimates to the actual tub dimensions. Still, our mesocosms were extremely productive. Max searched the literature for other high values (again note--before electronic libraries). He found a few examples of biomass as high as 15 kg/m<sup>2</sup>/yr for papyrus swamps in tropical Africa and cattails in Oklahoma. Our results were not entirely out of the ballpark, especially considering that San Diego has a year-round growing season for plants like California bulrush.

Remember our hypothesis, that alternating aerobic and anaerobic conditions would remove more N than constant conditions? Which hydroperiod treatment do you think maximized nutrient removal? How about heavy metal removal? Did we accept or reject our hypotheses? Max's and Theresa's MS theses and our joint publications told all (Busnardo et al. 1992; Sinicrope et al. 1992).

**TWICE-DAILY DRAINAGE WAS THE WINNER**. First, it caused the greatest nutrient removal: Compared to constant water levels, Max found 5-20% greater inorganic N removal and 20-30% more phosphate removal. With twice-daily drainage and lower N-loading, Max found 96% N-removal, and with twice-daily drainage and higher P loading, he measured 90% P-removal. The bulrush mesocosms were very efficient.

To our surprise, half of the N and P that we supplied was taken up and stored in the bulrush biomass. We had expected to find more of the P in the sediment, and more of the N denitrified and released to the air.

Second, Theresa found the highest metal removal with twice-daily discharge, regardless of loading rate. Average removal rates were

84% for lead, 75-78% for cadmium, chromium, copper and zinc, and 55% for nickel.

Most of the heavy metals that were removed were stored in the soil.

The results of our ambitious experiment supported our hypotheses that alternating wet and dry periods facilitate nutrient and heavy metal removal. Two more secrets revealed!

Wouldn't it be great if wastewater facilities would adopt our findings and build wetland treatment systems that retain water, remove N, remove heavy metals, and then release low-nutrient treated water? After Max began working as an environmental consultant, he developed a conceptual design for such a facility for a municipality that discharges treated wastewater to San Francisco Bay. It makes sense to

release nutrient-rich treated water on the ebb tide, so the nutrients are quickly moved to the ocean. The challenge is in building the capacity to store and then release large volumes of water in synchrony with outflowing tides.

Pulsed discharging was included in the master planning process, but the next step (adoption and implementation) still has to be taken. Costs are always a hurdle in going from great ideas to actual outcomes.

The "solution" at Tijuana Estuary was to discharge treated wastewater into deep water offshore, under the thermocline (boundary between warm surface water and cold subsurface water). Although nonsaline wastewater is lighter than seawater (so it should float), some of the heavier particles should sink or be used by marine plankton. Other materials are greatly diluted by seawater and picked up by currents and moved away. Together, these processes reduce the amount of material that floats back toward the shore.

**REALITIES AND THE NEED FOR INNOVATIONS**. Most engineered water-quality and waste-management facilities only reduce the amount of pollution; they do not eliminate problems. Wherever people live in high density, there are more wastes than can be removed or denatured downstream. Armed with that knowledge, don't you think that each one of us should minimize our individual impacts?

Re-using materials upstream reduces the need to manage wastes downstream. In my lifetime, I have watched litter-control programs help people stop tossing things along streets and highways; I have seen campaigns guide people to recycle plastics, paper and metal; I have noticed how manufacturers have shifted products toward biodegradable components, and that municipalities encourage water conservation. I was amazed when the City of Madison banned lawn fertilizers that include phosphorus! These trends are grounds for optimism (positive thinking).

Some people still toss their trash out the car window (I know; I spend hours picking it up along the road where I live); and some lawns still are fed far more nitrogen than they need to grow grass. So the next generation can work on fertilizer reduction, native-plant landscaping and gardening, xeriscaping, more use of permeable sidewalk and street materials, below-parking-lot storage of runoff during rainfall for re-use during dry periods, various catch basins and steppools instead of drainage ditches, and waste-reduction measures that have yet to be discovered and invented.

In short, we all need to follow a land ethic—to live in a place without spoiling it (Aldo Leopold). I'm counting on you to protect and restore our remaining salt marshes!