Created pools and food availability for fishes in a restored salt marsh

Daniel J. Larkin[a,*], Janelle M. West[b], Joy B. Zedler[a,c]

[a] *Department of Botany, University of Wisconsin-Madison, 430 Lincoln Dr., Madison, WI 53706, United States*
[b] *Department of Biological Sciences, Mira Costa College, 1 Barnard Dr., Oceanside, CA 92056, United States*
[c] *Arboretum, University of Wisconsin-Madison, 430 Lincoln Dr., Madison, WI 53706, United States*

ABSTRACT

Trophic support functions for fishes are a key goal of salt marsh restoration. Food availability in restored sites may be enhanced by creation of shallow pools, which are important sources of prey items in tidal wetlands. Young restored salt marshes are typically sparsely vegetated and are subject to rapidly changing geomorphology. Scouring and sedimentation create and fill shallow depressions, producing a shifting mosaic of tidal pools. In a large (8-ha) southern California experimental restoration site, we created shallow pools and assessed their development of foods for fishes. Created pools quickly developed abundant invertebrate prey, with densities exceeding those found in older, naturally formed pools ($P < 0.0001$). Opportunistic mobile and disturbance-associated taxa (calanoid copepods, nematodes, *Polydora* complex, and *Trichocorixa reticulata*) accounted for higher invertebrate densities in created pools. We repeated experiments in spring, summer, and fall and found seasonal variability in trophic development. We also applied bottom-up (nitrogen addition) and top-down (fish exclusion) treatments to pools. Some measures of algal biomass were increased by nitrogen fertilization ($P = 0.001–0.06$), but there were no upward-cascading effects on invertebrate composition or abundance. Fish abundance in the site varied seasonally, but there were no compelling effects of fish exclusion treatments on algal or invertebrate abundance. Incorporating shallow depressions into salt marsh restoration projects is a potential tool to jumpstart fish-support functions.

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1. Introduction

Ecological restoration seeks to create persistent, self-sustaining ecosystems (Allen et al., 2003), but young restored sites are vulnerable to disturbances that interfere with restoration goals (Holling, 1973; Suding et al., 2004). Improved understanding of recently restored sites' functional development can reduce uncertainty and increase achievement of restoration goals (Palmer et al., 1997; Kentula, 2000; Teal and Weishar, 2005). One important but often overlooked function of restored sites is trophic support for target fauna (Vander Zanden et al., 2006).

Salt marshes play an essential role in providing food for fishes (Boesch and Turner, 1984; Kneib, 1997) and trophic support functions are a key goal of salt marsh restoration (West et al., 2000; Weishar et al., 2005). In southern California, degradation or destruction of >90% of tidal wetlands have created a critical need for salt marsh restoration (Zedler, 2001) and many

large projects are underway or planned. Southern California marshes produce invertebrates and algae that are trophically important for *Fundulus parvipinnis* (California killifish) and *Gillichthys mirabilis* (longjaw mudsucker), the two fishes that commonly use marsh surfaces in the region (Kwak and Zedler, 1997; Talley, 2000; West et al., 2003; Larkin et al., 2008). West and Zedler (2000) found that *F. parvipinnis* with marsh access fed at rates six times greater and ate a more diverse suite of prey items than those restricted to subtidal channels. This increased feeding enhances *F. parvipinnis* growth and reproduction (Madon et al., 2001). *F. parvipinnis* is short-lived (∼12–18 months), with a highly seasonal life cycle (Fritz, 1975). The region's seasonally variable, mixed-semidiurnal high tides restrict when fishes can access marsh surfaces (Maloney and Chan, 1974; Madon et al., 2001). The food resources that *F. parvipinnis* encounters during these temporal "windows" of marsh access dictate the bioenergetic and reproductive benefits they garner from marsh utilization (Madon et al., 2001).

Recently restored sites are often sparsely vegetated and have fewer invertebrates than reference sites (Scatolini and Zedler, 1996; Armitage and Fong, 2004a). In such cases, shallow ephemeral pools on marsh surfaces can be important sources of invertebrate prey for fishes during high tides (Larkin et al., 2008), as they are in older restored and reference sites (Kneib, 1984; Angradi et al., 2001; Able et al., 2003). Over short time periods (hours to days), these pools fill with water and drain in response to tidal regimes. Over longer time periods, pools can form and disappear with seasonal oscillations between sediment resuspension and accretion (shown to change local surface elevation by ±13 cm in <1 year; Onuf, 1987; Ward et al., 2003; Wallace et al., 2005). This is particularly true in recently restored sites, where there is little vegetation to stabilize sediments and vegetation patchiness influences water flow (Fennessy et al., 1994; Wallace et al., 2005).

As tides and geomorphology interact to produce a shifting mosaic of pools, the biotic composition of these pools may be influenced by bottom-up and top-down trophic factors. Salt marshes generally have nitrogen (N) limited primary production (Sundareshwar et al., 2003) and N pulses have bottom-up effects on their plant, algal, and invertebrate communities (Fong et al., 1993; Boyer and Zedler, 1998; Gratton and Denno, 2003; Armitage and Fong, 2004b). Due to watershed urbanization, southern California estuaries often receive large pulses of N, especially during the rainy season (Boyle et al., 2004). Changes in consumer abundance can have reciprocal, top-down effects on salt marsh trophic resources (Quammen, 1981; Kneib and Stiven, 1982; Silliman et al., 2004). In southern California marshes, seasonal and long-term fluctuations in *F. parvipinnis* and *G. mirabilis* use of marsh surfaces (Larkin et al., 2008) may affect invertebrate abundance.

In an earlier study, we found that *F. parvipinnis* preferentially used naturally formed pool microhabitats, which had elevated food resources, over flat and vegetated portions of a young restored site (Larkin et al., 2008). In the present study, we experimentally created pools as a test of a potential trophic-function restoration tool. We assessed pools' development of food resources. We hypothesized that newly created pools would quickly develop food resources for fishes in excess of those found previously in flat, non-pool microhabitats (Larkin et al., 2008). We also expected created pools to differ from

older pools, with lower algal and invertebrate abundance and differing invertebrate composition (more mobile and disturbance-associated taxa; Netto and Lana, 1994; Scatolini and Zedler, 1996; Talley and Levin, 1999; Moseman et al., 2004; Zheng et al., 2004). We hypothesized that abundances of invertebrates and algae would vary seasonally (Brinkhuis, 1977; Zedler, 1982; Scatolini and Zedler, 1996; Caffrey, 2004). We expected N pulses (a common pollution event in highly urbanized southern California) to increase abundance of algae (Fong et al., 1993; Armitage and Fong, 2004b) and for increased algal biomass to cascade up to increase invertebrate abundance (Posey et al., 1995; Sarda et al., 1996). We hypothesized that predation effects of fishes on invertebrates would be negligible in spring and summer but detectable during the fall, when *F. parvipinnis* abundance peaks (Larkin et al., 2008).

2. Study area

The Tijuana River National Estuarine Research Reserve (Tijuana Estuary; 32°35′N, 117°07′W) is located in San Diego Co., CA, USA, on the international border with Mexico, wherein ∼75% of its 4500-km^2 watershed lies. The 1024-ha salt marsh-dominated estuary is in the Californian biogeographic region, with a Mediterranean-type climate (Zedler et al., 1992). Tijuana Estuary is important to regional biodiversity and, in 2005, was designated a Ramsar Wetland of International Importance (Ramsar Convention Bureau, 2006).

Tijuana Estuary is exposed to frequent disturbances due to its position in a densely populated watershed with highly erodible soils and development on steep, sparsely vegetated hillsides (Ward et al., 2003). After rainstorms, turbid, muddy, nutrient-rich water is discharged into the estuary (Greer and Stow, 2003; Ward et al., 2003; Boyle et al., 2004; Wallace et al., 2005). Nutrient loading is exacerbated by occasional overflows of sewage from a nearby treatment facility that serves Tijuana, Mexico (IBWC, 2006). There are also densely populated, unplumbed developments on the Mexican side of the watershed (RWBC, 2002). Pulses of nitrogen from raw sewage enter the estuary during rainfall events. A food web analysis of Tijuana Estuary found little evidence that sewage-derived organic matter directly influenced consumers but concluded that sewage may have indirectly influenced consumers by providing increased inorganic nitrogen to microalgae (Kwak and Zedler, 1997).

3. Methods

3.1. Experimental design

The Friendship Marsh in Tijuana Estuary was excavated in 1999 and opened to tidal flushing in February 2000 (Zedler et al., 2003). It is an 8-ha experimental salt marsh restoration site that contains three replicate "cells" (∼1.3 ha each) built with tidal creek networks and three replicates without tidal creeks (Entrix et al., 1991; Madon et al., 2002). Cells with and without creeks were paired in a complete block design, with the western half of each block including a creek network and the eastern half without. Cells were numbered 1–6 from west to

east. Each cell was constructed to have zones of unplanted mudflat (~0.3-m elevation NGVD 29, adjacent to a subtidal channel), an intermediate elevation with *Spartina foliosa* (Pacific cordgrass) planted as plugs, and a mostly unvegetated ~0.8-m elevation marsh plain with 108 plots experimentally planted with five halophyte species (Wallace et al., 2005).

Shallow pools formed on the marsh plain due to sediment resuspension from wind-driven waves (Wallace et al., 2005). Pools were variable in size (<0.1 to ~400 m^2) and maximum depth (<3 to ~20 cm) and conspicuous because of the marsh plain's low plant cover (Larkin, unpublished data). In 2005, marsh plain cover comprised primarily pools (46%), followed by vegetation (31%, dominated by the halophyte *Salicornia virginica*; Varty and Zedler, 2008). Pools generally contained elevated invertebrate abundance relative to non-pool areas (Larkin et al., 2008).

We created and experimentally manipulated pools on three occasions. We analyzed chlorophyll and invertebrate data to detect responses to season, N fertilization, and fish exclusion. Monitoring of natural pools and fish abundance was performed for a separate study (Larkin et al., 2008) and those data are cited herein to provide context for results of pool experiments.

3.2. Experimental pools

We performed pool experiments on three occasions. Treatments were applied using a 2 × 3 fully crossed factorial design testing the effects of N addition (control or fertilized) and fish exclusion (none, partial, or full). For each run of the experiment, we set up three blocks in a randomized complete block design. Each block consisted of 12 pools in a 2 × 6 matrix with 2 replicates of 6 unique treatment combinations (6 replicates and 36 pools per experiment). We sited all experimental pools in cells 5 and 6 to minimize confounding effects of spatial variability (i.e., distances between blocks and distances between experimental and natural pools). We arbitrarily positioned blocks in areas of the marsh plain that were unvegetated, relatively flat, and at intermediate elevation in the marsh plain.

We dug pools using a sharp-edged, bottomless plastic coring device and hand trowels. Each pool was circular in shape, flat-bottomed, 10-cm in depth (typical of natural pools), and had an area of 0.25 m^2. Pools were spaced 1 m apart on center. We randomly assigned treatments (full, partial, or absent exclosure; ±N addition) with stratification by block using R 2.5.1 (R Development Core Team, 2007). We placed three 5-cm × 5-cm unglazed ceramic tiles in each pool in a triangular formation to act as substrates for new growth of epibenthic algae. Tiles are highly durable media and are often used to assess algal growth in aquatic research (Steinman and Lamberti, 1996). We applied all treatments within 48 h of pool excavation.

For each pool receiving N fertilization, we evenly dispersed 22.4 g of NH_4–NO_3 fertilizer pellets (33% N by mass) in the pool for a loading rate of 30 g N m^{-2}. The same addition was repeated on the following day to compensate for tidal flushing. This loading rate is typical for nitrogen fertilization studies in salt marshes (Boyer and Zedler, 1998; citations therein).

For August and October experiments, fish exclosures were made of galvanized metal hardware cloth with 0.64-cm square mesh. Full exclosures consisted of 0.9-m tall circular fences with 2.25-m circumferences that completely enclosed pools and were designed to exclude fish while allowing movement of water and invertebrates. Partial exclosures were applied to test for unintended, confounding effects of exclosures (Connell, 1997). Partial exclosures were semicircles of hardware cloth that enclosed half of pools' circumferences and were positioned south of pools to maximize possible shading effects. In June, exclosures were constructed from plastic netting with 0.64-cm square mesh. While this material had been effective during an earlier pilot experiment, in June these exclosures became overgrown with algae and plastic netting was not used in subsequent months.

We created and treated pools during neap tides and harvested them (collected chlorophyll and invertebrates) during neap tides after two sets of spring tides had passed (each experiment ran for one lunar cycle or ~4 weeks). The first round of experimental pools was set up May 19–21 and harvested June 23–25 (fully exclosed pools were not sampled due to high algal growth on plastic netting used during that period). The second round was created July 22–25 and harvested August 22–23. The third set of pools was created September 25–26 and harvested October 21–22. Experimental cohorts are referred to hereafter by the month when they were harvested (June, August, and October).

3.3. Chlorophyll sampling and analysis

To sample epibenthic algae, we collected ceramic tiles from pools and immediately put them in plastic containers, which were then wrapped with aluminum foil and placed on ice. We sampled phytoplankton in pools by collecting ~100-ml water samples in plastic containers, also wrapped in foil and placed on ice.

We used spectrophotometric measures of chlorophyll (chl) concentrations as an indicator of algal abundance (APHA, 2005). Chl *a* is present in all photosynthetic algae, including cyanobacteria (blue-green algae), chl *b* is found in green algae, and chl *c* is found in photosynthetic diatoms (Rowan, 1989).

Chlorophyll samples were collected and processed on the same dates. Epibenthic algae were scraped off of tiles from experimental pools into centrifuge tubes. We vacuum-filtered water column chlorophyll samples through 0.45-µm pore size mixed cellulose ester membranes and transferred membranes to centrifuge tubes. We added 90% aqueous acetone (90 parts acetone, 10 parts saturated magnesium carbonate solution) to samples, sonicated them, and stored them in the dark for 12–24 h at 4 °C to extract pigments. We estimated biomass of chlorophyll *a* (with correction for presence of pheophytin, a chlorophyll degradation product), chlorophylls *b* and *c* by placing extracts in 1-cm quartz cuvettes and measuring optical densities with a Beckmann spectrophotometer. We tested extracts' absorbances at 750, 664, 647, and 630 nm prior to acidification and at 750 and 665 nm after 1.5-min of acidification with 1N HCl (APHA, 2005).

We calculated chlorophyll concentrations by area (mg m^{-2}) and volume (mg m^{-3}) for epibenthic and water samples, respectively. As is common at low chlorophyll concentrations, some negative values occurred as artifacts of measurement

error and biomass formulas. Negative values were changed to zeros and data were normalized using a log(x + 1) transformation prior to analyses. We analyzed data using a linear model in R 2.5.1 (R Development Core Team, 2007). We tested for differences among experimental pools by month and treatments.

3.4.   Invertebrate sampling and analysis

We collected epibenthic invertebrates using 3-cm deep, 7.5 (August, October) to 10-cm diameter (June) PVC cores. We placed cores in plastic containers, covered them with 90% ethanol, and shook the sealed containers so that the cores and ethanol would mix. We collected invertebrates in pool water columns by sinking a bottomless plastic bucket into the marsh surface. We then bailed water from the bucket and poured it through a 0.5-mm sieve. We washed samples with water, rinsed invertebrates from the sieve into plastic containers, and added 90% ethanol.

Invertebrate samples were soaked for 30 min in a Rose Bengal–ethanol solution to stain organic material. We then separated animals from debris and used a dissecting microscope (10–70×; Olympus Model SZH10) to count them and identify them to the lowest feasible taxon.

We calculated densities of invertebrates for epibenthic and water column samples as individuals m^{-2} and summed them to calculate total combined invertebrate densities. A log(x + 1) transformation was used to normalize data. We analyzed data using a linear model in R 2.5.1 (R Development Core Team, 2007). We employed multivariate methods to address differences in invertebrate density and composition. We calculated dissimilarity matrices for log-transformed invertebrate density data using Bray-Curtis distances between sample units. We performed non-metric multi-dimensional scaling (NMS) ordination and Analysis of Similarity (ANOSIM) testing using the vegan package in R 2.5.1 (Oksanen et al., 2006; R Development Core Team, 2007). We tested for differences between natural and experimental pools and differences among experimental pools by month and treatments.

4.   Results

4.1.   Block effects

Locations of experimental pools (block) significantly affected invertebrate densities ($P < 0.0001$) and concentrations of water column chl a ($P = 0.001$) and chl c ($P = 0.01$). Mean invertebrate densities differed greatly among blocks, ranging from 1262 ± 311 individuals m^{-2} (mean ± 1 S.E.) to 9856 ± 2534 in June, 435 ± 85 to 837 ± 175 in August, and 580 ± 79 to 3880 ± 646 in October. Concentrations of water column chl a varied from 99.1 ± 12.1 mg m^{-3} to 183.9 ± 13.9 in June, 10.3 ± 2.9 to 14.6 ± 4.1 in August, and 77.1 ± 21.5 to 378.5 ± 87.3 in October. Concentrations of water column chl c varied from 35.4 ± 5.2 mg m^{-3} to 58.4 ± 8.2 in June, 0.7 ± 0.5 to 3.7 ± 2.5 in August, and 19.4 ± 6.3 to 78.8 ± 18.1 in October. Concentrations of water column chl b and epibenthic chl a, b, and c were not affected by block ($P = 0.92$, 0.17, 0.49, and 0.37, respectively).
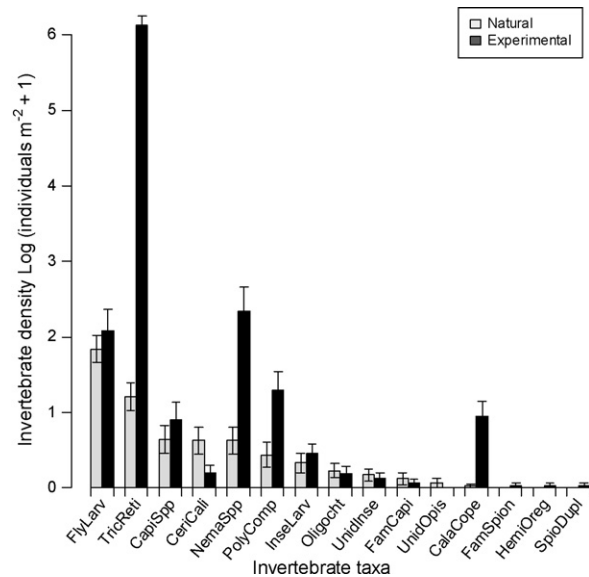

Fig. 1 – **Densities of invertebrate taxa found in naturally occurring and experimentally manipulated pools. Sampling of 36 experimental pools took place every 2 months spring-fall 2005. Data for natural pools are from Larkin et al. (2008) and are shown here for comparative purposes. Taxa codes: CalaCope, Calanoid copepod; CapiSpp, *Capitella* spp.; CeriCali, *Cerithidea californica*; FamiCapi, Family Capitellidae; FamiSpio, Family Spionidae; FlyLarv, Fly larvae; HemiOreg, *Hemigrapsus oregonensis*; InseLarv, Insect larvae; NemaSpp, Nematode spp.; OligSpp, Oligochaete spp.; PolyComp, *Polydora* complex; SpioDupl, *Spiophanes duplex*; TricReti, *Trichorixa reticulata*; UnidInse, Unidentified insect; UnidOpis, Unidentified opisthobranch.**

4.2.   Experimental pools vs. non-pools and natural pools

Invertebrate data from non-pool areas and naturally occurring pools in the Friendship Marsh are reported elsewhere (Larkin et al., 2008) and are included here for comparative purposes. Consistent with expectations, experimental pools had greater than two-fold higher densities of invertebrates than non-pools (1899 ± 319 individuals m^{-2} vs. 707 ± 160 individuals m^{-2}, respectively; $P < 0.0001$). Contrary to expectations, experimental pools also had higher densities of invertebrates than natural pools (1009 ± 218 individuals m^{-2}, $P < 0.0001$). There were no differences in mean taxonomic richness between experimental and natural pools (2.8 ± 0.1 and 3.1 ± 0.3 taxa per sample, respectively, $P = 0.30$) but ANOSIM testing showed that composition of experimental and natural pools differed significantly ($P < 0.001$). Invertebrate densities in experimental pools had higher proportions of disturbance-associated (nematodes, *Polydora* complex) and mobile taxa (*Trichocorixa reticulata*, calanoid copepods, Fig. 1).

4.3.   Seasonal variability

Experiment month influenced trophic structure, as indicated by differing abundances of algae and invertebrates (Fig. 2). Concentrations of epibenthic chl a, b, and c differed by month
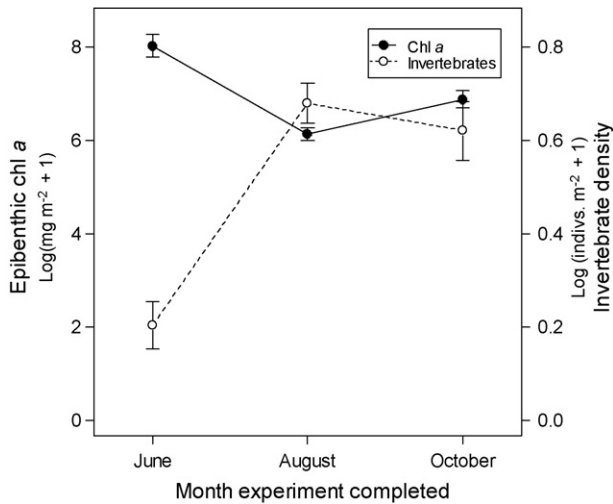
Fig. 2 – Relative abundances of invertebrates and epibenthic chl *a* by month in experimental pools.

Fig. 4 – NMS ordination of invertebrate data (epibenthic + water column) for experimental pools by month of experiment. Bray-Curtis dissimilarities were calculated using log-transformed density data and plotted using three dimensions (stress = 13.1). See Fig. 1 for species codes.

($P < 0.0001$, 0.01, and 0.0001, respectively), as did water column chl concentrations ($P < 0.0001$ for chl *a*, *b*, and *c*, Fig. 3). Epibenthic chlorophyll levels increased from spring to summer to fall. Water column chlorophyll levels were generally lower in summer than in spring and fall.

Experimental pool invertebrate densities varied by month ($P < 0.0001$, Fig. 1) and month was also a significant factor in multivariate analyses of invertebrate composition (ANOSIM test: $P < 0.001$, Fig. 4). Invertebrate densities were highest
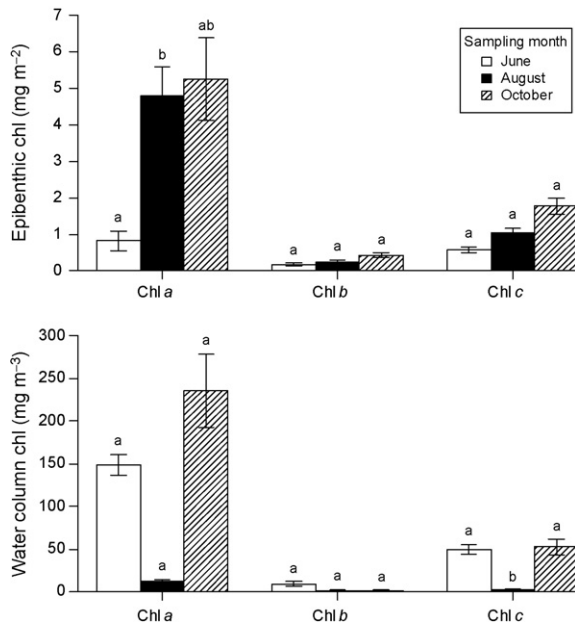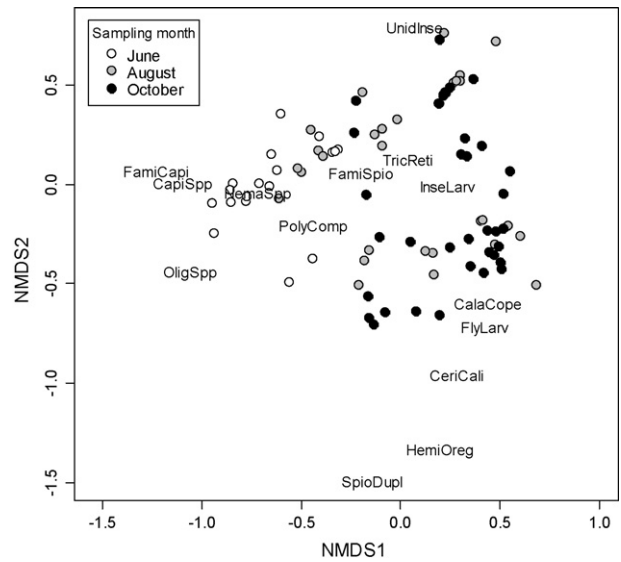
in June pools (4878 ± 1204 individual m^{-2}), lowest in August (614 ± 73), and intermediate in October (1689 ± 327).

4.4. Nitrogen fertilization effects

Epibenthic chl *a* and *c* concentrations showed a weak positive response to nitrogen addition ($P = 0.06$ and 0.04, respectively). However, chl *b* concentrations did not ($P = 0.28$). Water column chlorophyll response to nitrogen followed the same pattern (chl *a*: $P = 0.001$, chl *b*: $P = 0.68$, and chl *c*: $P = 0.01$, Fig. 5).

There was no evidence that nitrogen addition directly affected total invertebrate densities ($P = 0.61$) or invertebrate composition (ANOSIM: $P = 0.88$). There was moderate congruence between algal and invertebrate abundances in pools. Water column chl *b* and *c* concentrations had significant, positive associations with invertebrate densities ($P = 0.09$ and 0.008, respectively). However, the relationships were not strong ($R^2 = 0.10$ for chl *b* and 0.11 for chl *c*) and other measures of algal abundance (water column chl *a*, all epibenthic chl) had no significant correlations with invertebrate abundance ($P = 0.46$–0.97). Based on the design of this experiment, bottom-up effects of algae on invertebrates could not be untangled from possible reciprocal effects of invertebrate herbivory on algal biomass.

4.5. Fish exclosure effects

Detailed fish trapping results are reported in Larkin et al. (2008). For interpretation of fish exclosure effects, the following trends are notable: consistent with the known seasonality of *F. parvipinnis* life history, catch rates differed by month ($P < 0.001$) and were low during the spring and summer and higher in the fall. *G. mirabilis* catch rates also differed by month

Fig. 3 – Mean concentrations of epibenthic (top) and water column (bottom) chl *a*, *b*, and *c* in experimental pools. Error bars are ±1 S.E. Concentrations of each chl type differed significantly by month ($P = 0.01$ for epibenthic chl *b*, $P < 0.0001$ for all others). Within chl groups, different letters indicate significant pairwise differences between months ($\alpha \leq 0.05$, Fisher's test).
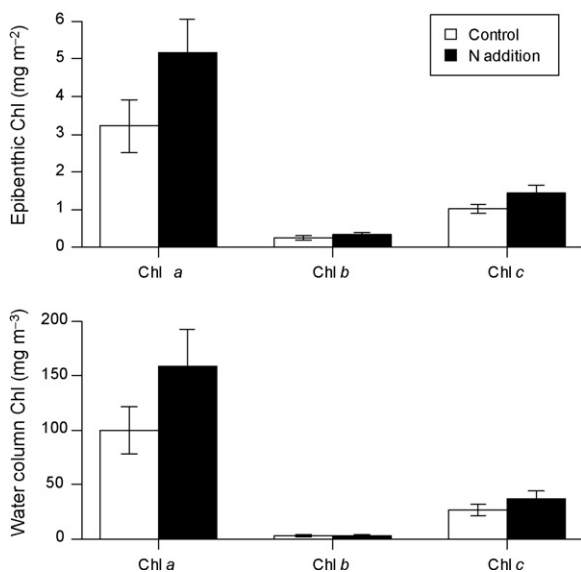
Fig. 5 – Mean concentrations of epibenthic (top) and water column (bottom) chlorophylls *a*, *b*, and *c* in experimental pools by N fertilization treatment. Error bars are ±1 S.E.

($P < 0.001$) and were highest in early summer and lowest in late summer. *F. parvipinnis* were caught at nearly nine-fold higher rates in traps deployed in pools than those deployed on flat ground ($P < 0.001$), despite the fact that all trapping occurred when the entire marsh was inundated by high tides.

Fish exclosures had no effect on epibenthic chl *a*, *b*, or *c* concentrations ($P = 0.51$, 0.72, and 0.22, respectively). Exclosure type was a significant factor in water column concentrations of chl *b* and *c* ($P = 0.07$ and 0.03, respectively) but not *a* ($P = 0.31$). For water column chl *b*, concentrations were highest in the open (no exclosure) treatment (mean ± 1 S.E.: $4.4 \pm 1.4 \, \mathrm{mg \, m^{-3}}$), intermediate in the partial exclosure treatment (1.9 ± 0.9), and lowest in the fully exclosed treatment (1.8 ± 1.0). Water column chl *c* concentrations followed the same pattern, with open, partial, and exclosed means of 33.4 ± 5.3, 32.0 ± 10.2, and 29.3 ± 10.3, respectively.

Exclosure type did not affect total invertebrate densities ($P = 0.11$) but there was weak evidence that exclosure treatments affected composition and abundance of invertebrates (ANOSIM: $P = 0.078$).

5.   Discussion

Experimentally created pools quickly developed food resources for fishes. From gut content and stable isotope analyses, we know that the invertebrate taxa found in created pools are suitable prey for *F. parvipinnis* and *G. mirabilis* (Kwak and Zedler, 1997; West et al., 2003; Kwak unpublished data; Madon unpublished data). Invertebrates were higher in abundance and had differing composition than were found in a companion study of non-pools and naturally occurring pools (Larkin et al., 2008). Algal abundance increased with nitrogen fertilization but invertebrates were not influenced by nitrogen addition (bottom-up) or fish access (top-down) treat-

ments. Pool creation may be a useful tool for jumpstarting fish-support functions in young, restored sites.

5.1.   Experimental pools differed from natural pools and other sites

As expected, invertebrate densities in created pools exceeded those found in non-pools. We expected young pools (~4 weeks old) to have *lower* densities of invertebrates than existing pools, but this was not the case. A few taxa accounted for the high densities in created pools (Fig. 1); these were opportunistic passive deposit feeders (*Polydora* complex, nematodes) and mobile arthropods (*T. reticulata*, calanoid copepods) often found in disturbed settings (Euliss et al., 1991; Netto and Lana, 1994; Levin et al., 1996; Hart et al., 1998; Schratzberger et al., 2004).

In addition to created pools' newness, their morphology was a likely factor in differences observed between created and natural pools. The area of created pools ($0.25 \, \mathrm{m^2}$) was within, but on the low end of, the range found for naturally occurring pools (~0.1–$400 \, \mathrm{m^2}$). Depth of created pools ($10 \, \mathrm{cm}$) was typical of that found for natural pools but created pools were steep-walled rather than sloping and had lower area-to-depth ratios. It is likely that these differences in created pool morphology influenced algal, invertebrate, and fish responses; as the character of topographic heterogeneity, not just its presence or absence, has been shown to influence biotic responses (Vivian-Smith, 1997; Larkin et al., 2006).

Overall, the densities of invertebrates we found were much lower than in other salt marsh studies. Talley and Levin (1999) sampled invertebrates in marsh plain portions of four restored salt marshes in southern California and found densities ranging from 28,000 to 290,000 individuals $\mathrm{m^{-2}}$. In contrast, the highest monthly mean we encountered was <5000 individuals $\mathrm{m^{-2}}$. Lower invertebrate densities in our study are likely due to larger sieve mesh size, a younger site, and lack of vegetation cover in sampled areas (Scatolini and Zedler, 1996; Talley and Levin, 1999). Moseman et al. (2004) also found higher invertebrate densities in the Friendship Marsh (26,000 individuals $\mathrm{m^{-2}}$) than in our study but their sampling took place in a *Spartina*-vegetated area near the main tidal channel. Invertebrate assemblages differ between *Spartina* and marsh plain habitats (where we sampled), and comparisons are problematic (Talley and Levin, 1999). Also, the cores taken by Moseman et al. (2004) were sieved with smaller-sized mesh (0.3 mm rather than 0.5 mm), which would lead to more smaller taxa being identified. The cores of Moseman et al. (2004) were also 6 m deep while ours were only 3 cm deep (as our focus was on invertebrates readily accessible to fishes).

Water in pools is exchanged frequently via tidal flushing, so we expected phytoplankton levels to vary with sampling time and to be less likely than epibenthic algae to show treatment effects. However, water column chlorophyll concentrations were significantly increased by N addition, indicating that tidal flushing is insufficient to "reset" conditions in pools. This finding also suggests that there may be exchanges between the benthic and pelagic algal components of pools.

Epibenthic chl *a* concentrations in our study were orders of magnitude lower than those of other manipulative experiments in southern California salt marshes (Armitage and

Fong, 2004b; Boyer and Fong, 2005). Variability in algal biomass is high in southern California salt marshes, complicating comparison between sites (Zedler, 2001). For example, Armitage and Fong (2004b) and Boyer and Fong (2005) both sampled chl *a* in Mugu Lagoon, yet their results differed greatly. Southern California's mild climate, the presence of nitrogen-fixing cyanobacteria, and high spatiotemporal variability from tides might reduce environmental controls on algal assemblages and increase the influence of stochastic processes.

5.2. Temporal variability

Algal abundance and invertebrate composition and abundance differed significantly with month of experimentation. Patterns of epibenthic chlorophyll concentrations were consistent with expectations, increasing from spring to summer to fall during those periods' favorable growth conditions (Zedler, 1980, 1982). Water column chlorophyll concentrations also followed the predicted pattern, showing a mid-summer decline in phytoplankton abundance. This is consistent with elevated tidal amplitudes during this period (NOAA, 2006), which can lead to dilution of phytoplankton, and net ecosystem metabolism of Tijuana Estuary being negative (respiration in excess of production) during the summer (Caffrey, 2004).

Invertebrate densities did not follow the predicted temporal pattern of increase from spring-fall, instead showing a decline in summer. Salt marsh invertebrate abundances can be highly variable over time, making patterns difficult to discern (Kneib, 1984; Levin and Talley, 2002; West et al., 2003), even with long-term monitoring (e.g., 11 years; Desmond et al., 2002). Elevated tidal amplitudes prior to sampling of July pools may have flushed out some invertebrates. It is also possible that spatial variability (block effects) confounded temporal patterns.

5.3. Bottom-up effects

As expected, epibenthic algal abundance was increased by nitrogen addition (based on measurements of chl *a* and *c*). This was also the case for water column chlorophyll concentrations, despite frequent tidal water exchanges. Our finding agrees with other studies that implicate nitrogen as a limiting nutrient in salt marshes (Fong et al., 1993; Valiela et al., 1997; Boyer and Zedler, 1998; Gratton and Denno, 2003; Armitage and Fong, 2004b).

We did not see a significant effect of nitrogen addition on invertebrate composition or abundance. While algal abundance increased with fertilization and invertebrates were positively associated with some measures of algal abundance, there was no upward cascade of nutrient effects. The combination of high mobility in some invertebrate taxa (e.g., *T. reticulata*) and small (0.25 m^2) experimental units may have led to low fidelity of invertebrates to fertilized areas. It is also possible that the experiments were too brief to show nitrogen effects on invertebrates. Another salt marsh study showed attenuation of a potential cascade from algae to invertebrates and a negative herbivore response to fertilization (Armitage and Fong, 2004b).

5.4. Top-down effects

We found no compelling evidence of top-down effects from fish exclosures. There was weak evidence of exclosure effects on water column chl *b* and *c* and invertebrate composition. However, the lack of an invertebrate density response to exclosures leads us to conclude that these effects were spurious or unintended effects of exclosures (Connell, 1997). One confounding effect of exclosures might have been greater attraction or retention of invertebrates due to enhanced vertical structure. This could have led to increased herbivory in such pools and reduced chlorophyll concentrations (the pattern we saw). However, this also should have been accompanied by an effect of exclosures on invertebrate density. Shading effects seem more likely, as this would explain the high to intermediate to low chlorophyll abundance pattern observed with open, partial, and exclosed treatments, respectively. It is also possible that a leachate from the hardware cloth (e.g., zinc) reduced algal growth. When plastic netting was used, the opposite problem arose: overgrowth of algae. The close proximity of pools (1.0 m apart on center) was necessitated by the limited availability of sufficiently large, flat, unvegetated areas. However, these short distances could have led to carry-over effects between adjacent pools with different exclosure treatments.

It is also possible that fish use of the marsh did not occur with adequate frequency or at sufficient densities to offset stronger spatial, temporal, and biotic variables. In addition, the unique morphology of experimental pools (see above) may have made them unattractive to foraging fishes.

Characteristics of salt marshes make them difficult habitats in which to detect top-down effects. They are very open systems, with not just strong but, through tide flow and ebb, *bi-directional* connections to adjacent habitats. Salt marshes are dynamic and complex, with high abiotic variability, and can be affected by subtle differences in habitat heterogeneity (Adam, 2002; Larkin et al., 2008). These and other factors are likely to buffer top-down effects, turning potential cascades into trickles (Strong, 1992). As Strong (1992) notes, "spatial and temporal heterogeneity are not a prominent part of true trophic cascades, which are processes operating homogeneously in space." Recent field studies showing strong top-down effects and trophic cascades in salt marshes (Silliman et al., 2004, 2005) have occurred in systems dominated by large, monotypic stands of *Spartina*, making them relatively spatially homogenous. They have involved organisms with tight trophic linkages (i.e., periwinkle snails that exert strong top-down control on *Spartina*, blue crabs that are major predators of periwinkle snails). Also, the predators involved (crabs and a terrapin) were resident marsh organisms, with less temporal variability in abundances than fish, which come and go with the tides. Kneib and Stiven (1982) demonstrated top-down control of invertebrates by *Fundulus heteroclitus*. However, their study involved keeping manipulated densities of fishes in a marsh through the use of enclosed water-holding pits to enable survival through low tides. This "constant fish" treatment differs markedly from natural conditions in southern California, where semidiurnal mixed tides lead to brief and infrequent marsh inundations,

limiting the accessibility of marsh surfaces to fishes (Larkin et al., 2008).

5.5. *Restoration recommendations*

Previous work in southern California has indicated that naturally formed pools function as "oases" of elevated food abundance that are preferred microhabitats for *F. parvipinnis*, an important target fish species for salt marsh restoration (Larkin et al., 2008). Studies in other regions have demonstrated trophic and other benefits of pools to fishes (Smith and Able, 1994; Angradi et al., 2001; Able et al., 2003; Stevens et al., 2006).

The present study demonstrated that pools created to mimic a potential restoration tool quickly developed abundant food resources for fishes. Particularly in young, sparsely vegetated marshes in early stages of invertebrate colonization, pools added as restoration treatments may accelerate the emergence of fish trophic support functions. Factors likely to influence prey availability for fishes from created salt marsh pools, and which may warrant further study, include seasonal timing of pool creation as well as pool elevation, area, depth, and morphology.

6. Conclusions

Consistent with prior findings that increased habitat heterogeneity led to increased abundances of opportunistic invertebrates (Netto and Lana, 1994), we found that newly excavated pools quickly provided fish prey and in higher abundances than in older pools naturally formed by sediment resuspension.

The timing of pool formation influenced the abundance of algae and abundance and composition of invertebrates that colonized pools. Nitrogen addition affected the abundance of algae but not the abundance or composition of invertebrates. We found no compelling effects of fish exclosures on invertebrate densities. Brief and temporally variable tidal inundations, seasonality in fish abundances, and potential confounding effects of exclosures may have reduced detectability of top-down effects from fishes.

Incorporating shallow pools into salt marsh restoration projects can accelerate the development of trophic support functions. Larger-scale, longer-term experiments and tests of how created pools' sizes and morphologies influence their trophic development would advance our understanding of this potential tool for restoration of trophic support.

Acknowledgments

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