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Functional Diversity and Composition of Microalgae and Photosynthetic Bacteria in

Marine Wetlands: Spatial Variation, Succession, and Influence on Productivity

A dissertation submitted in partial satisfaction of the

requirement for the degree Doctor of Philosophy

in

Oceanography

by

Christopher Nathan Janousek

Committee in charge:

Lisa Levin, Chair
Paul Dayton
William Fenical
David Holway
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2005

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University of California, San Diego

2005

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DEDICATION

This dissertation is dedicated to my parents, Joseph and Pennie Janousek, and to my

parents-in-law, Charmayne Replogle and the late Brian Replogle (1946–1990).

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LIST OF ABBREVIATIONS

AI	assimilation index, a measure of productivity (in μg C fixed chl a^{-1} s^{-1})
B_3, B_4, B_5	microphototroph community biomass, based on the total concentration of the pigments fuco, lute, zeax (B_3); or fuco, lute, zeax, and bchl a (B_4); or fuco, lute, zeax, peri and bchl a (B_5)
bchl a	bacteriochlorophyll a
bchl b	bacteriochlorophyll b
bchl a/chl a	bacteriochlorophyll a/chlorophyll a (in μg μg^{-1})
bph a	bacteriopheophytin a
car	carotenes
chl a	chlorophyll a
CRK	intertidal creek-bank habitat
diad	diadinoxanthin
diat	diatoxanthin
DISSIM	dissimilarity (in %) between treatments, based on multivariate measures of composition
fuco	fucoxanthin
HPLC	High Performance Liquid Chromatography
I	irradiance (in μE m^{-2} s^{-1})
lute	lutein
MBAY	Mission Bay
MIX	non-*Spartina foliosa*-dominated salt marsh
MUD	intertidal mudflat habitat

NAT	natural wetland
NS	not significant (usually $p > 0.05$)
nMDS	non-metric multi-dimensional scaling
PDA	photo diode array detector
P	photosynthetic production (in μmol O_2 l^{-1} s^{-1})
REST	restored wetland
sf	solvent front
SIM	similarity (in %) within a treatment, based on multivariate measures of composition
SPAR	*S. foliosa*-dominated salt marsh
TIES	Tijuana Estuary
zeax	zeaxanthin
zeax/fuco	zeaxanthin/fucoxanthin (in μg μg^{-1})

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VITA

1975	Born, Fremont, California
1994-1996	Missionary, the Church of Jesus Christ of Latter-day Saints, Japan
1999	B.A., *Marine Biology*, University of California, Santa Cruz
1999	Phi Beta Kappa election, University of California, Santa Cruz
2003	Teaching Assistant, University of California, San Diego
2005	Ph.D. *Oceanography*, University of California, San Diego

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The success of any work such as this is only possible because of the efforts of numerous past and present scholars who have prepared the way for further advances; so accordingly, recognition should first be extended to them. For me, credit for my own immediate foundation in marine botany must be given to Dr. Lynda Goff (UC Santa Cruz) for her enthusiastic introduction to marine plants. Her course initiated a deep fascination with the marine producers (particularly of central California) and cast a spell that eventually lured me away from planned graduate work on invertebrates. Dr. Lisa Levin, my advisor, introduced me to wetland ecology, the study of wetland microproducers, and some of the ecological and statistical techniques I have employed to study these organisms. She provided general and specific guidance throughout the process and very promptly, and with care probably not extended by many academics, reviewed my writing. Other faculty members at UC Santa Cruz and Scripps have provided the other important building blocks of this dissertation and my general education. Drs. Ralf Goericke and Maria Vernet were instrumental in my acquisition of the major analytical technique of this dissertation: pigment analysis by High Performance Liquid Chromatography. Additionally, Dr. Wiebke Ziebis (USC) generously trained me on the utilization of oxygen microelectrodes while she was a post-doctoral researcher at SIO. I thank my other committee members, Drs. Dayton, Fenical, Holway and Knowlton for their scholarly insights and inspiration and their contributions to an improved dissertation.

Many people at SIO and several undergraduates at UCSD and at other institutions assisted with the field and laboratory work that helped my research progress. Among these are Christine Whitcraft, Joel Fodrie, Elizabeth Frame, Jessica Nolan, Lyra Pierotti, Evan Green (UC Berkeley), Janessa Stream (Columbia U.), Noah Ben-Aderet, Carlos Neira, Guillermo Mendoza and Jesse Leddick. I especially thank Christine and Jesse for their significant collaboration and contributions. Dr. Lilian Busse and Dr. Carolyn Currin assisted with the identification of diatoms and cyanobacteria, respectively.

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Finally, engagement in any work of natural discovery should enhance our appreciation and admiration for the marvel of the natural world around us. Indeed, the frantic cast of our time should not be allowed to divert us from the sublime and enriching show that nature provides in her silently, but persistent way. I acknowledge

the sea plants, large and small, studied and unstudied, who hold such a significant fraction of my interest. May we prudently protect the fragile fringe of shallow ocean that these creatures own so that their secrets might be revealed to us long into the future. I concomitantly acknowledge He who has made such marvels of biodiversity a part of the mortal experience while we are here on earth.

The geographic features of Figure 3.1 and several references throughout the dissertation were obtained from federal government works (see appropriate reference lists for exact federal agency). To the extent that any of that material represents a derivative work of public domain material, it may also be in the public domain and thus be free of copyright protection.

The material in Chapters I and Chapter V have been prepared for joint publication as a review article. Additionally, Chapter II, Chapter III, Chapter IV, and Appendix 1 have been prepared for publication as independent articles. The dissertation author is the sole author and principle investigator on all material as it appears here.

ABSTRACT OF THE DISSERTATION

Functional Diversity and Composition of Microalgae and Photosynthetic Bacteria in

Marine Wetlands: Spatial Variation, Succession, and Influence on Productivity

by

Christopher Nathan Janousek

Doctor of Philosophy in Oceanography

University of California, San Diego, 2005

Lisa Levin, Chair

Phylogenetically diverse benthic producers (e.g., diatoms, green algae, cyanobacteria, and anoxygenic photobacteria) inhabit the sediments of coastal wetlands and mediate numerous ecosystem functions, including a substantial fraction of total ecosystem productivity. Despite their ubiquity and functional importance, spatio-temporal patterns in the distribution of major taxonomic groups and diversity-related effects on function are poorly understood. Using a photopigment-based approach I investigated the composition of microproducer communities at two coastal sites in southern California and addressed (1) the temporal development of assemblages in a restored wetland, (2) the spatial distribution of major functional groups across various habitats, and (3) the influence of functional group diversity and dominance on primary productivity.

Within a large restoration site at Tijuana Estuary, pigment concentrations suggested rapid recovery of assemblage biomass (<1 yr) and at least partial recovery

of taxonomic composition by 0.2 yr in restored mudflats. In *Spartina*-dominated salt marsh, however, composition and diversity took longer to mirror natural marsh communities (~1.5-2.2 yr). In Mission Bay, study of an older restoration site (6.3 yr) also suggested differences in rates of habitat development, with *Spartina* marsh and creek-banks probably preceding mixed species marsh. In a spatial analysis of communities, I found that natural sediments from vegetated marshes in Mission Bay were enriched in cyanobacteria and anoxygenic photobacteria, and had higher diversity, relative to unvegetated sediments, but no mudflat versus marsh difference was evident at Tijuana Estuary. In several independent studies of diversity and productivity, including a manipulation of functional richness in lab cultures, I found no effect of variation in functional diversity on rates of oxygenic photosynthesis and only minor increases in microproducer standing stocks in more diverse assemblages. However, results also tentatively suggest unique community roles for green algae (high oxygenic production rates) and anoxygenic photobacteria (enhancement of community biomass), supporting the notion that composition may be more important than absolute diversity to function.

These data re-enforce the idea that study of composition in microproducer assemblages is necessary in addition to simple measures of chlorophyll a. The functional consequences of altered diversity and composition should be further investigated in this cryptic but important flora.

Chapter I.

Algae and photosynthetic bacteria in marine wetlands:

the community ecology of benthic phototrophs

1

Introduction

Shallow-water sedimentary environments fringing marine coasts throughout the world host non-vascular plant producers such as eukaryotic algae (diatoms, green seaweeds), cyanobacteria, and other photosynthetic prokaryotes (Blum 1968, Sullivan and Currin 2000). These organisms often appear inconspicuous, but they are ubiquitous components of the floras of shallow subtidal (Cahoon 1999), and intertidal sedimentary habitats such as salt marshes, estuarine mudflats, and sandflats (Sullivan and Currin 2000). Microproducer communities (also termed "microphytobenthos" or "microbial mats") are associated with inorganic sediment particles as well as living and detrital vascular plant material (Stowe 1982, Barnhart et al. 1992, Currin and Paerl 1998). Because of their microscopic nature and the corresponding challenge of comprehensive ecological study on these organisms, for many years microproducers were overlooked in certain aspects of wetland ecosystem and community ecology. Within the last few decades, however, ecologists have begun to recognize the breadth of ecological functions they support and their key position within the functional mosaic of coastal ecosystems.

Much of our current ecological understanding of marine wetland microphytobenthic communities centers on two of their (related) key functions - primary production and support of higher trophic level consumers. Macroalgae (mainly green algae and *Vaucheria*), microalgae (diatoms, euglenoids), and prokaryotic producers (cyanobacteria, anoxygenic phototrophic bacteria) contribute to carbon fixation in coastal salt marshes and mudflats via both oxygenic (O_2 – evolving) and anoxygenic (H_2S, etc.-based photo-oxidation) photosynthesis. Algal/photobacterial versus vascular plant contributions to total ecosystem

production appear to vary geographically. In salt marshes of the eastern United States, usually about 25-33% of total productivity can be attributed to algae (Marinucci 1982, Sullivan and Currin 2000). However, in other tidal marshes, microproducer carbon fixation can equal the contribution made by vascular plants (Zedler 1980, Fejes et al. 2005). Production by various macroalgae (e.g., Chlorophyta, Phaeophyta) in marine wetlands may also be considerable (Roman et al. 1990). In coastal California, estimates of algal and photobacterial contribution to total estuarine or total wetland productivity span 30 to ~50% (Zedler 1980, Barnhart et al. 1992).

Because of stable isotope research conducted over the last two decades, ecologists have discovered that a large portion of microphytobenthic production ends up fueling coastal wetland food webs. $\delta^{13}C$ and $\delta^{15}N$ concentrations in primary and secondary wetland consumers studied from a variety of geographic locations suggests that a substantial fraction of higher trophic level carbon may be derived from algal foods (Sullivan and Moncreiff 1990, Currin et al. 1995, Kwak and Zedler 1997, Page 1997, Kurota et al. 2001, Kang et al. 2003). Wetland grazers include protozoa, nematodes, oligochaetes, copepods, gastropods, amphipods, and fishes (Admiraal 1984). Additionally, production of carbon by microalgal populations may support the growth of heterotrophic bacteria (Cammen and Walker 1986). Although microphytobenthic biomass may be small compared with vascular plant standing stocks in certain wetland habitats, algal foods may be more palatable and nutritious to consumers than vascular plant material (Miller et al. 1996). Together, stable isotope studies lend much support for a more comprehensive perspective of wetland food webs in which the key role of microproducers is recognized (Figure 1.1).

⟵ *Carbon flow*

⟵ - *Nutrient flow*

Birds

Fishes

Benthic/pelagic invertebrates

Heterotrophic bacteria

1,4

4

1,4

Benthic microproducers & seaweeds

Vascular plants

2

Phytoplankters

3

3

2,3

Atmosphere/seawater
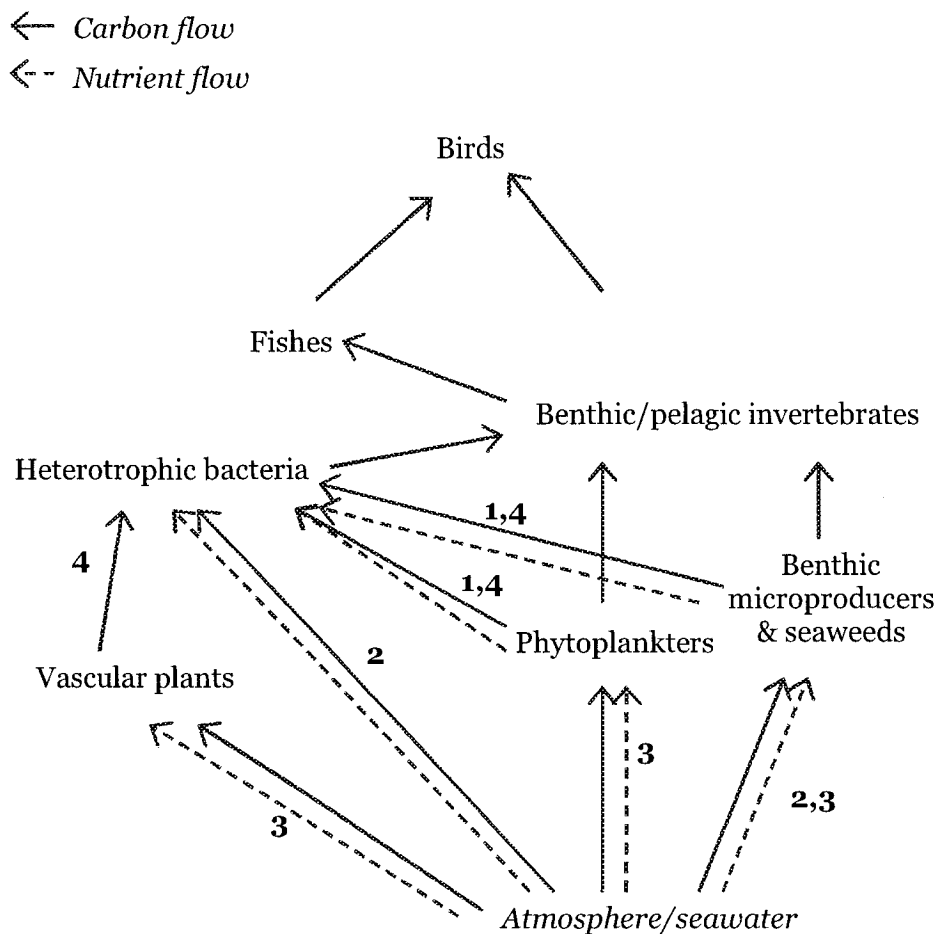
Figure 1.1. A simplified food web model for temperate marine salt marshes (a mudflat food web would be similar except for substitution of vascular plants with plant detritus). Significant pathways of carbon and nutrient (mainly N) advection are depicted for each trophic level and functional guild. Nutrient fluxes are depicted only for more basal components of the food web. Wetland algae and photosynthetic bacteria can mediate a substantial portion of ecosystem energy flow, but their relative contribution will vary according to season (e.g., Sundbäck et al. 1996), geographic location (Stribling and Cornwell 1997), and other factors. Processes: "**1**" leakage of extracellular carbohydrates from microphytobenthos, "**2**" nitrogen fixation, "**3**" nutrient uptake, "**4**" decomposition. All other pathways represent direct consumption. References: Ho (1974), Buffan-Dubau and Carman (2000), Naeem (2002).

Many investigators have sought to understand spatial and temporal variation in microproducer biomass and productivity. These studies show biomass and/or productivity differences associated with the physical structure of sediments (Plante et al. 1986), with vascular plant type (Sullivan and Moncreiff 1988), or according to geographic location (Pinckney et al. 1995a). Biomass and productivity can also vary seasonally (Leach 1970, Underwood and Paterson 1993b, Gall and Blanchard 1995) and during the course of a day (Van Raalte et al. 1976) as different taxa migrate to the sediment surface (Underwood et al. 2005). A wide variety of factors have been shown by experimentation, or inferred by correlative approaches, to influence standing stocks or photosynthetic rates including irradiance (Van Raalte et al. 1976), salinity (Sullivan and Moncreiff 1988b, Pinckney et al. 1995a), and grazing (Armitage and Fong 2004). For example, biomass is often positively correlated with intertidal height (Underwood and Paterson 1993b, Brotas et al. 1995) and smaller sediment particles (Davis and McIntire 1983).

While understanding of spatial and temporal variation in microphytobenthic abundance, productivity, and contributions to coastal food-webs has improved over the past several decades, gaining a more robust perspective on microphytobenthic ecology requires elucidation of variability in the biological *composition* and *diversity* of communities of these organisms. Changes in the diversity and composition of communities may be linked to the effects of abiotic and biotic disturbance on assemblages (e.g., Sousa 1979, 1984) and may be important to understanding spatial and temporal patterns of ecosystem processes (Loreau et al. 2001). In particular, several major topics need to be addressed in wetland microproducer assemblages: (1) What is the magnitude of spatial and temporal variation in composition and diversity

across different scales? (2) Which biological and environmental factors structure the composition and diversity of communities? (3) What are the physiological and ecological responses of individual functional groups to environmental variation? (4) What are the principle biological interactions between species and functional groups (e.g., competition, herbivory, facilitation) that shape community composition? (5) What effects do changes in diversity and function have on ecosystem-level processes? and finally, (6) How do temporal/spatial patterns, physiological responses to environmental change, and functional responses interact to shape ecosystem-wide dynamics? Such a conceptual model of ecological understanding, with diversity at its core, may help guide our future research on wetland microproducers. The majority of this dissertation addresses questions 1 and 5 in detail, but results pertinent to question 2 are also given.

Spatial variation in microphytobenthic composition and diversity

The study of spatial heterogeneity in the composition of communities is pre-eminent in benthic ecology. Like their macroalgal counterparts, benthic marine microproducers exhibit compositional variation across elevation zones of the intertidal (Javor and Castenholz 1981, Saburova et al. 1995, Zong and Horton 1998), between various landscape features (Sullivan and Moncreiff 1988), and at small microscales (Blanchard 1990). It is important to keep in mind that these communities show spatial structure both horizontally across the sediment surface (from mm to 1000 km scales) and vertically through sediment layers (at the μm to mm scale). Small-scale (cm to m) horizontal patchiness may be an important, and possibly a common, feature of microphytobenthic distribution (e.g., Pinckney and

Sandulli 1990). Casual observation in the field, for example, suggests the presence of distinct patches of cyanobacteria or green algae that extend from mm to m over the sediment surface (Figure 1.2). Additional studies suggest that horizontal heterogeneity in community structure appears to grow as the spatial scale of investigation grows (Pinckney and Sandulli 1990, Nübel et al. 1999, Safi 2003). Interestingly however, research also suggests a high degree of compositional taxon overlap between geographical areas separated by hundreds to thousands of kilometers (Sullivan and Currin 2000). Such apparent floristic homogeneity may reflect high rates of dispersal between wetland habitats on large spatial scales (perhaps by oceanographic and/or animal vectors), consistent with the paradigm of a high degree of cosmopolitanism in microbial taxa (Finlay and Clarke 1999). Alternatively, apparent large-scale floristic similarity from a morphological perspective may mask true regional differences in microalgal genotypes because morphology might underestimate genetic diversity (e.g., Taton et al. 2004).
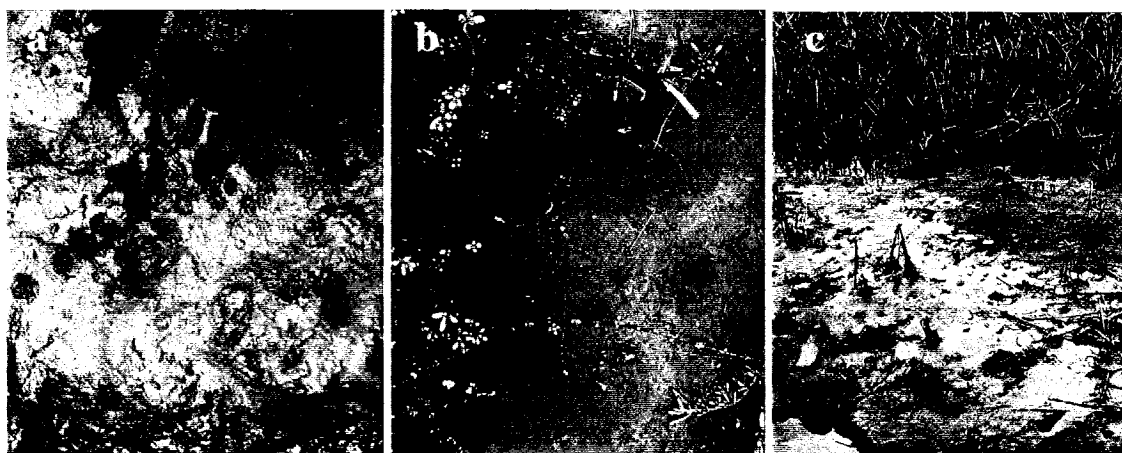
Figure 1.2. Wetland patches of (a) sediment microproducers and (b, c) macroalgae at Mission Bay. Vascular plants are *Batis maritima* (in b) and *Spartina foliosa* (in c).

In addition to horizontal variation in communities, a large fraction of ecological research on microproducers has been dedicated to the study of vertical microgradients found in the upper layers of sediments. Algae and photosynthetic bacteria inhabit thin layers of sediments (the top few mm) that are characterized by steep gradients of declining oxygen and light (Garcia-Pichel et al. 1994), and higher sulfide with depth (Stal 2000). These microgradients provide stratified layers of different physio-chemical conditions and appear to determine microproducer zonation. For example, a generic depth profile from the water column into wetland sediments might reveal a surface covering of green macroalgae, an upper sediment layer of diatoms, a deeper layer of cyanobacteria, and then a final stratum of anoxygenic photobacteria (Pierson et al. 1990). These latter organisms inhabit the elusive vertical zone characterized by sufficiently high irradiance, low oxygen, and enough sulfide for anoxygenic photosynthesis (Underwood and Kromkamp 1999).

Despite the many studies that have examined microproducer variation across geographical space, certain gaps in our understanding remain. For example, most studies have focused on community variation within a single taxonomic group (e.g., diatoms), so it is still poorly understood how the relative abundances of major taxonomic groups (cyanobacteria, diatoms, green algae, tribophytes, euglenoids, phototrophic bacteria) are allocated across space. Additionally, there is little information about which major features of wetlands induce shifts in the composition of major functional groups. Some research suggests that changes in light (Sullivan 1976) or nutrients (Camacho and de Wit 2003) may affect the relative abundances of cyanobacteria versus diatoms, but additional work shows that these factors have little effect on functional composition (Sullivan 1981, Sundbäck and Snoeijs 1991). More

manipulative experimental work needs to be conducted to better pinpoint the factors and mechanisms that determine spatial heterogeneity.

Temporal change and succession in microproducer communities

Microphytobenthic communities are known to vary on seasonal scales (Sullivan and Moncreiff 1988, Pinckney et al. 1995b, Sullivan and Currin 2000), but most of this research has focused on variability in production or biomass. Zedler (1982) noted seasonal shifts in some microphytobenthic taxa at Tijuana Estuary in southern California, and Currin and Paerl (1998) found seasonal changes in the taxon composition of cyanobacterial taxa present on *Spartina* shoots. Less in known about seasonal variation in the composition of major functional groups, but a single year of observations by Pinckney et al. (1995b) showed a rise in cyanobacteria abundance relative to diatom biomass during summer months and a shift to greater diatom dominance during the winter.

Little work has been conducted on variation in community composition or diversity that may be present at larger (>1 yr) temporal scales. Stal et al. (1985) noted a change in cyanobacterial community composition (from *Oscillatoria*-dominated to *Microcoleus*-dominated assemblages) at a single site in the North Sea over the course of approximately 2.5 yr. Underwood's (1997) study of recovery of microphytobenthic communities in an English salt marsh restoration project also showed a shift from *Oscillatoria* and *Spirulina* to *Microcoleus*-dominated communities at a lower intertidal marsh station, but little compositional change over time at other vegetated and unvegetated sampling locations. Peletier (1996) found long-term changes in

diatom community species composition in a simple (species-poor) mudflat system in central Europe after a reduction of anthropogenic nutrient inputs into the estuary. There has been no published long-term (>5 yr) monitoring of variation in any undisturbed salt marsh habitat and minimal published work dedicated to a study of long-term community succession associated with large-scale disturbances or restoration activities.

Even though microphytobenthic populations experience very rapid growth (Williams 1964, Underwood and Paterson 1993a), and might be expected to change principally on short time scales, longer-term temporal variation in these communities cannot be ignored. A better understanding of ecosystem processes and health, for example, requires more information on longer-term variability in community composition and diversity in response to such phenomena as eutrophication (e.g., Peletier 1996), sedimentation, and habitat alteration. It is also desirable from a management perspective to ascertain if successional dynamics differ by geographic region, sediment characteristics, wetland habitat size, or habitat structural complexity.

Microproducer diversity and function

In the early 1940's Edith Purer conducted a botanical inventory of the wetland flora of San Diego County. Her documentation of the local producers and work on the natural history of wetland plants drew her to this conclusion with regards to the non-vascular plant flora: "Algae play an insignificant role in these marshlands." (Purer 1942). In the intervening decades, ecologists have learned much that runs counter to this statement. In particular, algal and bacterial producers are now known to be

central to the biogeochemical cycles of carbon and nitrogen in wetlands. Various eukaryotic algal producers in wetland sediments conduct oxygenic photosynthesis, providing food for wetland consumers and oxygenation of surface sediments (Baillie 1986). All purple and green photobacteria fix carbon anoxygenically and many cyanobacteria (undoubtedly including some wetland taxa) are also capable of anoxygenic production via sulfide oxidation (Garlick et al. 1977). Additionally, the physical presence and carbohydrate secretions of benthic microproducers confer stability to surface sediments (Grant and Gust 1987, Austen et al. 1999), and aid in sediment accretion (Coles 1979).

Because no two taxonomic groups of microproducers are physiologically or behaviorally equivalent, changes in the composition and/or diversity of microproducers could influence aggregate ecosystem functions. At higher taxonomic levels for example, there is substantial variation in the kinds of metabolic processes conducted by different microphytobenthic groups (Table 1.1). In particular, there is an especially pronounced disparity between the functional capabilities of prokaryotic producers (which mediate biogeochemically-important reactions such as sulfide oxidation and nitrogen fixation) and the more metabolically- limited eukaryotic producers. Of course, functional heterogeneity may also exist at finer taxonomic scales as well. For example, differences in sulfide tolerance and anoxygenic photosynthetic production (Cohen et al. 1986) and nitrogen fixation (Castenholz et al. 2001) exist across different species (and even among different strains of the same species) of cyanobacteria.

Despite stated interest in diversity-productivity relationships within benthic microproducer assemblages (Underwood and Kromkamp 1999, Underwood et al. 2005), there has been no large-scale attempt to study diversity effects on what is

Table 1.1. Physiological and behavioral characteristics of benthic microproducers occurring in wetlands. ●: major function or function found in most taxon members. •: minor function or function limited to only some taxon members. p: probable function but uncertain. References: (1): Admiraal 1984, (2) Calvo and Bárbara 2004, (3) Ehrenreich and Widell 1994, (4) Grant and Gust 1987, (5) Imhoff 1992, (6) Kondratieva et al. 1992, (7) Madigan 1988, (8) Pfennig 1989, (9) Polderman 1978, (10) Stal 2000, (11) Sundbäck and Snoeijs 1991.

Taxonomic group	Representative genera	Oxygenic photosynthesis	Anoxygenic photosynthesis	Photoheterotrophy	Organoheterotrophy	Fermentation	Nitrogen fixation	Sulfide oxidation	Iron oxidation	Self-propulsion	Sediment shading	Sediment stabilization	Silicate uptake	Exopolymer secretion	References
Diatoms	*Navicula*	●			•					●	•	●	●	●	1
Euglenoids	*Euglena*	●								●					9
Green algae	*Ulva, Rhizoclonium*	●									●			p	11
Tribophyceae	*Vaucheria*	●									•	●			9, 2
Cyanobacteria	*Oscillatoria, Gloeocapsa*	●	•	•		•	●	•		•		●		•	6,8,10,11
Photosynth. bacteria	*Thiocapsa, Chromatium*		●	•	•		●	●	•	●		●			3,4,5,7

Physiological, behavioral, or ecological characteristic

arguably the most important function of wetland microproducers – carbon fixation. Sullivan and Moncreiff (1988) briefly mention a negative association between carbon fixation rates and diatom species richness in *Spartina* salt marsh, but there is no comprehensive published account of how genetic, species, generic or functional group diversity influences community-level rates of production or standing stocks of microproducers.

Scope of the dissertation

The observations and experiments described in this dissertation represent a step towards a deeper understanding of spatial and temporal variability in, and the functional consequences of, community composition and diversity of wetland microproducers. In *Chapter II*, I investigate differences in community composition and functional diversity across different marine wetland habitats defined by vascular plant composition and geomorphology at two southern California sites. I also compare community composition and diversity between restored and natural wetlands within each site and test for differences in composition at the site level (Mission Bay versus Tijuana Estuary). These data provide insight into variability in microproducer diversity and composition at spatial scales of great importance to local management and conservation efforts.

In *Chapter III*, I track microphytobenthic succession in a newly restored wetland in southern California and investigate differences in the tempo of recovery between *Spartina foliosa* salt marsh and unvegetated mudflat. I also characterize spatial differences in assemblage patches within the restored site and examine relationships between community abundance and composition with environmental

features. This study is the first comprehensive account of multi-year wetland microproducer succession at the functional group level.

The field and laboratory studies described in *Chapter IV* focus on biodiversity and functional group dominance impacts on primary production and standing stocks in assemblages of benthic algae and phototrophic bacteria. I use field-collected communities and laboratory cultures to examine functional richness and functional group diversity (richness+evenness) effects on gross oxygenic photosynthesis and relationships with standing stocks. I also use pigment communities obtained across a variety of dates at two sites to test if diversity and biomass relationships in microphototroph communities differed between restored and natural wetlands.

In *Chapter V*, I discuss the novel insights of each of these studies and how they compare with our understanding of the community ecology of wetland microproducers gained from other research. The discussion culminates in a conceptual model of how functional composition and diversity of microproducers are structured in, and influence the processes of, marine wetland ecosystems. I mention considerations for successfully establishing microproducer communities in wetland restoration projects. *Appendix 1* provides a preliminary account of microphytobenthic diversity within southern California wetlands and *Appendix 2* describes techniques of sediment pigment analyses.

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Chapter II.

Spatial heterogeneity in the abundance, composition, and functional diversity

of microalgae and photosynthetic bacteria in marine wetlands

Abstract

The sediments of coastal marine wetlands host communities of phylogenetically diverse microproducers such as diatoms, cyanobacteria, and anoxygenic photosynthetic bacteria, but little is understood about spatial variability in the major taxonomic groups forming these assemblages. Using taxon-specific photosynthetic pigments, I investigated differences in phototroph biomass, composition, and functional diversity between (1) different intertidal habitats and (2) restored and natural wetlands at Tijuana Estuary and Mission Bay in southern California. I also compared the functional group composition of Tijuana Estuary versus Mission Bay assemblages. Within Mission Bay creek-banks and *Spartina* salt marsh, composition and functional diversity were similar between natural and restored wetland but natural marshes hosting mixed vascular plants had higher microproducer diversity and more cyanobacterial biomass relative to diatoms than restored marsh. In Tijuana Estuary sediments, microproducer abundance, composition, and diversity were similar between natural and restored wetlands and between mudflat and *Spartina* marsh, except for a natural-restored difference in the abundance of anoxygenic bacterial producers. Only slight compositional heterogeneity was evident between the two sites. In Mission Bay, a shift in prokaryotic to eukaryotic producer abundance appears to be linked to higher organic matter, but no other measured sediment characteristics seemed to influence diversity or composition. This research suggests that variation in microphototroph communities was present at a variety of scales in the coastal wetlands studied, but that the scale of variability may be site dependent. With greater understanding of the spatial distribution of microproducers, wetland ecologists are better situated to

evaluate, and plan for, the success of coastal restoration efforts and to predict spatially-linked changes in ecosystem function.

Introduction

Coastal marine wetlands in temperate southern California comprise various tidally-influenced landscapes and sites of different ages and histories. Mudflats, intertidal channels, high intertidal salt pannes, and salt marsh vegetated by salt-tolerant vascular plants are common to the region and occur in spatial mosaics organized by geologic forces, tidal and hydrologic influence, natural disturbance regimes, ecological interactions between vascular plants, and coastal development (Redfield 1972, Callaway and Pennings 2000). Much of the wetland habitat in southern California present in early historic times has been subsequently lost or heavily influenced by human alteration of coastal areas (Marcus 1989, Zedler et al. 2001). Habitat degradation has led to, among other effects, decreases in local biodiversity (Ibarra-Obando and Poumian-Tapia 1991, Zedler et al. 2001). To compensate for lost aerial coverage, a number of wetland restoration plans have been implemented in the southern California region (Zedler 1996).

In tidal marine wetlands, benthic algae and phototrophic bacteria form a functionally important component of salt marsh and mudflat biota (Miller et al. 1996). These organisms include benthic diatoms, cyanobacteria, anoxygenic phototrophic bacteria, euglenoids, green algae, and the benthic tribophyte, *Vaucheria* (Palmisano et al. 1989, Pinckney et al. 1995, Sullivan and Currin 2000). Most of the organisms in this component of the wetland flora are present as microscopic forms (and are collectively known as "microphytobenthos" or "benthic microalgae"), but

these producers are neither exclusively microscopic nor algal in nature. Although microphytobenthic assemblages are often treated as a cohesive group in ecological studies, communities of these phototrophs represent rich phylogenetic diversity with substantial variation in metabolic, physiological, and behavioral characteristics. This variability leads to the diverse functional roles played by microphytobenthic assemblages in addition to carbon fixation via primary production: nitrogen fixation (Carpenter et al. 1978), sulfide oxidation (Stal 2000), nutrient flux regulation (Tyler et al. 2003), sediment stabilization (Austen et al. 1999), and trophic support of metazoans (Page 1997). In this study, smaller macroscopic green algae were (generally) included in the communities studied, but "microphytobenthos" and related appellations have been retained in reference to these assemblages.

Standing stocks (biomass) of sediment-dwelling benthic algae and photosynthetic bacteria may vary temporally and across a suite of spatial scales. Chlorophyll a is routinely measured to determine standing crops, but this measure estimates only the biomass of eukaryotic algae, cyanobacteria, and sediment-deposited plant detritus. A number of studies have found spatially-linked differences in chlorophyll a abundances that correspond to either gradients of elevation(Brotas et al. 1995) or specific wetland habitats (Sullivan and Moncreiff 1988, Pinckney and Zingmark 1993). Additionally, Plante et al. (1986) observed small (cm) to large (km) scale variation in microbial mat phototroph biomass that was associated with changes in hydrologic (wave intensity) and substrate features. Quantifying spatial variation in biomass is vital for understanding grazing and sedimentation effects in wetland ecosystems and for predicting contributions of microalgae to ecosystem productivity.

However, aggregate measures of microproducer communities (such as estimation of chlorophyll a) fail to capture variation in the composition or diversity of

assemblages - features of communities that may be important to ecosystem function (Loreau et al. 2001, Zedler et al. 2001). The relatively limited work on spatio-temporal differences in microphytobenthic composition and diversity in wetlands (Hagerthey et al. 2002) usually centers on seasonal variability in communities (Underwood 1994, Pinckney et al. 1995), or explores spatial variation only within a single large taxonomic group (e.g., Sullivan 1975, 1977, Sullivan and Sage 1978, Currin and Paerl 1998; but see Zedler 1982). Little is known about how major functional (taxonomic) groups are distributed spatially in marine wetlands.

Much of the spatial variation in composition investigated to date has involved microphytobenthic community differences across elevation zones. For example, Javor and Castenholz (1981) noted differences in salt marsh cyanobacterial composition across intertidal zones (Javor and Castenholz 1981). Using rRNA estimates of diversity, Rothrock and Garcia-Pichel (2002) found lower diversity in assemblages of heterotrophic bacteria and cyanobacteria at higher intertidal elevations. Underwood (1994, 1997) also noted elevational gradients in diatom diversity and the densities of several microphytobenthic species. However, additional work at the species level suggests that sediment microproducer taxa are generally quite evenly distributed across intertidal elevations (with only differences in abundance across zones), suggesting widespread dispersal and/or broad tolerance of a variety of intertidal conditions (Stewart and Pugh 1963, Sage and Sullivan, Zedler 1982, Saburova et al. 1995). In a study of microphytobenthos present in salt marsh and hypersaline ponds, Nübel et al. (1999) found little variability in microphytobenthic rRNA "richness" at very small (1-2 dm) scales, but saw increased heterogeneity at stations located roughly 1 km apart.

Research with a more mechanistic focus on community heterogeneity suggests

that diversity and composition differences may be driven by environmental gradients found in intertidal wetlands or by biotic interactions with other wetland organisms. For example, in some cases, disturbance may play an important role in determining levels of diversity (Underwood 1997, Rothrock and Garcia-Pichel 2002), and higher salinity has been shown to be associated with lowered diatom diversity (Clavero et al. 2000). Changes in the particle size, salinity, and compaction of sediments (Underwood 1997, Zong and Horton 1998, Waterman et al. 1999), metazoan grazing (Hagerthey et al. 2002, Armitage and Fong 2004), nutrient concentrations (Van Raalte et al. 1976, Pinckney et al. 1995), and/or shading of the sediment surface by vascular plants (Sullivan 1976, 1981) may potentially influence the presence and abundance of particular microphytobenthic species or functional groups in wetland sediment assemblages.

In this chapter I present a pigment-based study of differences in benthic phototroph community abundance, composition, and functional diversity at a suite of spatial scales in southern California wetlands. I compared several wetland habitats (mudflat, creekbanks, mixed halophyte marsh, *Spartina* marsh) and natural and restored ecosystems in two coastal locations (Mission Bay and Tijuana Estuary) in southern California. I tested the null hypotheses that sediments hosted similar abundances, similar taxonomic composition, and equivalent functional diversity of microproducers between (1) different habitats and (2) restored and natural wetlands. I also tested for compositional heterogeneity between Mission Bay and Tijuana Estuary communities. Finally, within Mission Bay habitats, I investigated relationships between abiotic conditions of the sediment environment (light, salinity, moisture, grain size, and organic matter) and the composition and functional diversity of benthic producers.

Materials and methods

The northeastern corner of Mission Bay contains remnant salt marsh from a previously more extensive system that covered much of the bay (32°47' N, 117°13' W; Purer 1942, Levin 1982). A small, <3 hectare, mitigation site was constructed in 1995 (Levin and Talley 2002) and is separated from adjacent natural habitat by an elevated berm but is hydrologically connected to natural wetland via a single second order channel. Salt marsh within both restored and natural areas is vegetated by halophytes such as *Salicornia bigelovii* Torrey, *S. virginica* Linn., *Batis maritima* Linn., *Jaumea carnosa* Gray, *Limonium californicum* Heller, *Suaeda esteroa* Ferren and Whitmore, *Frankenia salina* (Molina) Johnston and *Triglochin* sp(p)., but areas of nearly pure *Spartina foliosa* Trin. are also present. Both restored and natural wetland habitat contains first and higher order intertidal creeks. Natural salt marsh is separated from the subtidal waters of Mission Bay by an expanse of unvegetated mudflats.

Tijuana Estuary is located just north of the U.S./Mexico border in southern California (32°34' N, 117°7' W). It is one of the largest remaining wetlands in the region but has also been reduced in extent over the last two hundred years (Zedler 1996). The estuary contains salt marsh dominated by *S. foliosa* or mixtures of other halophytes such as *Salicornia* spp. Small to moderate-sized channels and expanses of unvegetated mudflat situated between creeks and salt marsh are also present. A large wetland restoration site (8 ha) was created adjacent to natural salt marsh and opened to tidal influence in February 2000 as part of a longer term attempt to increase aerial coverage of intertidal wetland (Zedler et al. 2001). The restoration site was initially composed of mudflat habitat bisected by a region of planted *S. foliosa* with additional

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transplants of other plant species (Zedler et al. 2001).

Fifty-six intertidal wetland locations were randomly selected from a working map of intertidal habitat at Mission Bay and sampled during 26-29 March 2002. The following habitats were targeted: unvegetated mudflat (MUD), unvegetated creek banks (CRK), *Spartina*-dominated salt marsh (SPAR; roughly ≥80% *Spartina* biomass), and salt marsh with one or more other halophyte plant species (MIX; < about 20% *Spartina* biomass; see Figure 2.1). All mudflat locations were situated within approximately 200 m of the land-ward edge of the marsh. MUD, CRK, SPAR, and MIX habitats are roughly situated along a gradient of increasing tidal elevation respectively (Zedler 1977). Eight locations were sampled from all habitats at both the natural and restoration sites (except mudflat which is not found conterminous with the restored marsh). At each location, cores of surface sediment were taken for analysis of photosynthetic pigments and sediment water capacity (0.85 cm diameter, 1 cm depth), sediment grain sizes and organic matter (~1.4 cm diameter, ~1-2 cm depth), and salinity of interstitial water (~1.4 cm diameter, several cm depth). At a subset of locations, light transmission through the plant canopy to the sediment surface was measured with a QSL-100 light meter (Biospherical Instruments Inc.) by dividing light levels near the sediment surface with incident irradiance approximately 1 m above the marsh floor.

From 7-8 April 2002, 48 sediment communities at the Tijuana Estuary restoration site and adjacent natural marsh were collected as part of a longer-term study of wetland succession (see Chapter III). Twelve replicate locations within natural and restored wetland were haphazardly chosen in both MUD and SPAR habitats. Sediments of a brownish color were usually targeted for sampling in Tijuana Estuary, including the exclusion of areas with apparent macroalgal cover, whereas

MUD

Figure 2.1. Wetland habitats targeted in this study. MUD=mudflat, CRK= creek-bank, SPAR=*Spartina* salt marsh, MIX=mixed halophyte salt marsh.

macroalgae, if present, were included in Mission Bay cores. Surface sediment for pigment analysis, sediment interstitial water salinity, and water content were collected similarly to that described for Mission Bay except that pigment and water cores consisted of two replicate cores taken to 0.5 cm depth. All sediments were frozen (-80°C for pigments, -20°C for others) following collection.

At both sites, *S. foliosa* shoot densities were obtained in SPAR habitat by counting living and non-living plants of greater than approximately 10 cm height inside 0.25 m² or 0.0625 m² plots. Sediment pore water salinity was estimated in the field on a refractometer after pressing wet sediment through filter paper set inside a

plastic syringe. Since four salinity samples from the restored SPAR habitat in Tijuana Estuary exceeded 100 psu, (the limit of the refractometer) estimates of 100 or 105 psu were used. The water retention of sediments was obtained by measuring water loss (change in weight) after drying at ~55-60°C. Sediments collected for organic matter and particle size (% sand) analyses were separated though a 63 μm sieve into large (sand + granules) and small (silt + clay) fractions. Each fraction was dried, weighed, and then treated with ≤30% hydrogen peroxide for up to a few months to oxidize organics (Carver 1971). Generally, after little evidence of further reaction, sediments were washed, dried, and reweighed. Total organic matter was expressed as the combined weight loss (in mg) of both fractions following H_2O_2 treatment. The proportion of sand was calculated as the mass of inorganic particles exceeding 63 μm divided by total inorganic particle mass.

Photosynthetic pigment abundances in surface sediments were quantified using High Performance Liquid Chromatography (e.g., Palmisano et al. 1989, Brotas and Plante-Cuny 2003). Frozen sediment was defrosted and an appropriate volume of 100% acetone was added to form a 90% acetone solution for pigment extraction. (The volume of acetone for each sediment core was calculated based on estimation of sediment water content). Pigments were extracted at ~0°C for ~24 hr. Following centrifugation, supernatants were filtered through a cotton-plugged glass Pasteur pipette (Goericke 2002) and collected in clean containers. 100 μl aliquots of 600 μl supernatant + 250 μl water mixtures were prepared for separation by an Adsorbosphere® C_{18} HS reverse-phase column (3 μm stationary phase, 10 cm length, Alltech Associates, Inc.) coupled to a C_{18} HS guard column (5 μm stationary phase) and 0.5 μm in-line filter. Solvent was delivered by a Waters® 600E pump according to one of two gradients modeled after Brotas and Plante-Cuny (1996). Both gradients

('F' and 'H') utilized 80% methanol and 20% 1.0M ammonium acetate in water [phase 1] and 70% methanol/ 30% ethyl acetate [phase 2] mixtures delivered at 1.0 ml min^{-1} over 40 min. Gradient 'F' proceeded from 100% phase 1 /0% phase 2 at the beginning to 0% phase 1/100% phase 2 from 30-37 min, and a return to the initial composition by 40 min. Gradient 'H' differed from 'F' only at t=0 min where mobile phase composition began with 70% phase 1/30% phase 2. Absorbance of chlorophylls and carotenoids was measured at 450 nm on a Spectra 100 variable wavelength detector; chlorophylls were also detected using a Waters® 470 fluorescence detector (excitation=430 nm, emission=674 nm).

For determination of bacteriochlorophyll a concentrations, additional 100 µl aliquots (diluted with water as above) were analyzed on a third gradient ('G') modeled after Goericke (2002). Separation occurred on an Adsorbosphere® C$_{18}$HS reverse-phase column (5 µm stationary phase particles, 4.6 cm diameter, 15 cm length) coupled to a pre-column (5 µm stationary phase) and 0.5 µm pre-filter with two mobile phases (phase 1: acetonitrile/water [60%/40%] and phase 2: acetone) distributed over a gradient as follows: 0 min: 80% phase 1/20% phase 2, 1 min: 50% phase 1, 10-14 min: 0% phase 1/100% phase 2, and 16m: 80% phase 1/20% phase 2. Detection was by absorbance at 770 nm. For gradients 'F' and 'H', elution times of sediment pigments were compared with those of single species analyzed periodically (Table 2.1). Bacteriochlorophyll a elution time was confirmed by comparison with semi-pure bacteriochlorophyll a obtained from *Rhodopseudomonas sphaeroides* (Sigma-Aldrich, Inc.). For pigments obtained on all gradients, detector responses were converted to µg pigment per unit area of surface sediment (µg cm^{-2}), as is standard in microphytobenthic research, by using calibration equations generated with pure pigment standards, the original volumes of 90% acetone used in

extractions, and the surface area of sediment collected in the field.

Chlorophyll a (chl a) and bacteriochlorophyll a (bchl a) concentrations were used as estimates of oxygenic and anoxygenic phototroph biomass respectively (Pinckney et al. 1995) and are given per-unit area of surface sediment (in $\mu g\ cm^{-2}$). The carotenoids fucoxanthin (fuco), lutein (lute), and zeaxanthin (zeax) were used to quantify the abundances of diatoms, green algae (+plant detritus) and cyanobacteria (Table 2.1; Pinckney et al. 1995, Jeffrey et al. 1997). The taxonomic (functional) composition of communities was estimated by three separate measures: (a) bchl a/chl a ratios for anoxygenic versus oxygenic phototroph biomass, (b) zeax/fuco ratios for cyanobacteria versus diatom biomass (Pinckney et al. 1995) and (c) multivariate characterizations of communities based on the abundances of fuco, zeax, bchl a and lute normalized to the total mass of all four taxon-specific pigments (B_4=fuco+zeax+lute+bchl a). Finally, functional group diversity was calculated with four diagnostic pigments using Simpson's diversity index ($1/D_4 = 1/\sum p_p^2$ where p_p= pigment/B_4), a measure incorporating variation of both evenness and richness (Magurran 2004).

Reliable estimation of phototroph biomass by environmental pigment concentrations can be complicated by pigment changes at the cellular level due to variation in irradiance (Falkowski and LaRoche 1991) and nutrient concentrations (Geider et al. 1988). Thus, comparison of chl a or bchl a concentrations between unvegetated and vegetated habitat, for example, (high light differences) must be viewed with caution. However, restored versus natural comparisons, wherein light differences are not likely to be substantial, are probably not heavily influenced by photoacclimation effects. Moreover, because fuco and zeax (Brotas and Plante-Cuny 2003), and chl a and bchl a (presumably) respond similarly to changes in irradiance,

Table 2.1. Distribution of photosynthetic pigments in the wetland algae and photobacteria of interest in this study. Gradients 'F' and 'H' were derived from Brotas and Plante-Cuny (1996); gradient 'G' was after Goericke (2002). Approximate elution times are given because of minor extract to extract and day to day variation. ^ Estimated.

Taxonomic group	Diagnostic pigment	Approx. elution (min)			material used for elution time	Reference(s)
		F	H	G		
diatoms	fucoxanthin	12.5	5.8	-	*Thalassiosira weissflogii*	Pinckney et al. 1995
green algae/plants	lutein	20.1	15.9	-	*Dunaliella tertiolecta*	Jeffrey and Vesk 1997
cyanobacteria	zeaxanthin	20.4^	16.2	-	sediment/*Spirulina*	Pinckney et al. 1995
oxygenic phototrophs	chlorophyll a	28.8	26.5	-	*Dunaliella tertiolecta*	Pinckney et al. 1995
anoxy. phototrophs	bacteriochlorophyll a	-	-	12.3	bacteriochlorophyll a	Pinckney et al. 1995

the measures of community composition should not be heavily influenced by habitat-level differences in light.

Variation in microphytobenthic communities was examined at a hierarchy of spatial scales. The comparisons of interest were between (1) wetland *habitats* (MUD, CRK, SPAR, MIX), (2) wetland *status* (natural versus restored) and (3) wetland *sites* (here, only multivariate differences in microphytobenthic composition between Mission Bay and Tijuana Estuary were considered). Differences in individual environmental properties such as salinity and sediment water capacity and the pigment measures described above were tested across habitats and wetland status (scales 1 and 2) for each site separately using fixed-factor two-way ANOVA (CoStat 6.101). Null hypotheses were rejected at $p < 0.05$. The pigment composition of sediment communities at each site (habitat and status scales) were graphically represented with non-metric multi-dimensional scaling (nMDS) procedures generated from a Bray-Curtis similarity matrix based on measures of dominance of each functional group (p_p) (see Beals 1984). Heterogeneity in composition between habitats and between natural and restored wetland was tested separately at each site (experiment-wise $\alpha = 0.025$) with ANOSIM using ≥ 1000 bootstraps (a multivariate ANOVA analogue; Primer 5.2.2 software). Additionally, site-level differences in composition were tested with ANOSIM on pooled Mission Bay and Tijuana Estuary communities from *Spartina* marsh and mudflat. Within and between-treatment similarities and dissimilarities for all multivariate analyses were determined with SIMPER (Primer 5 software). Communities from Mission Bay mudflats were omitted from two-factor ANOVA tests to create balanced analyses of variance (Quinn and Keough 2002), but were included in figures, in multivariate comparisons, and in

regression analyses (described below).

The potential influence of abiotic environmental factors on community composition and diversity (zeax/fuco, bchl a/chl a, and $1/D_4$) was explored using linear correlations for Mission Bay assemblages. The following factors were included as abiotic variables: sediment moisture at low tide, sediment pore water salinity, proportion of sand in sediments, proportion of organic matter in sediments, and light transmission to the sediment surface. Abiotic variables generally did not exhibit strong correlations with each other in pair-wise comparisons (visual inspection). Separate groups of correlations were conducted for restored and natural wetland assemblages. Abiotic and pigment measures were transformed as needed (e.g., arc-sine for proportions; Sokal and Rohlf [1995]) to improve normality and/or homogeneity of variances, but where non-normal distributions were still present, rank correlation analyses were used.

Results

Spatial variation in sediment environments. There were a number of distinct abiotic differences according to wetland habitat and status. Sediment pore water salinities in Mission Bay wetlands differed across habitats ($F_{2,42}=4.2$, p=0.022), but not between restored and natural wetlands ($F_{1,42}=3.8$, p=0.059; Table 2.2). Elevated salinities in the restored MIX marsh relative to natural MIX sediments drove a significant habitat-status interaction ($F_{2,42}=3.3$, p=0.047). In contrast, sediment moisture at low tide showed little habitat-level differences ($F_{2,42}=2.4$, p=0.100), but natural sediments retained significantly more water than restored wetlands ($F_{1,42}=92.4$, p<0.0001). Natural sediments also contained substantially more organic

Table 2.2. Spatial differences in sediment environmental variables (means±S.E.[n]) in Mission Bay (above) and Tijuana Estuary (below) according to wetland habitat and status (natural versus restored). Significance levels for each site-specific 1 or 2-factor ANOVA are given below means (a posteriori Tukey-Kramer tests at $\alpha<0.05$). In Mission Bay, mudflat locations were excluded from the ANOVAs. *Spartina* densities are in plants m^{-2} ($\geq$10 cm height); light transmission in proportion of irradiance at the sediment surface. Habitats: C=Creeks, S=*Spartina* marsh, M=mudflat and X=mixed halophyte salt marsh. NA=not applicable or not measured.

Site, status, habitat		*Spartina* density	Salinity (psu)	Water (g g^{-1})	Sand (g g^{-1})	Organics (g g^{-1})	Light trans.
MISSION BAY Natural	Mudflat	NA	41.8±1.6 (8)	0.48±0.03 (8)	0.46±0.08 (8)	0.04±0.01 (8)	0.94±NA (1)
	Creek-bank	NA	41.9±2.4 (8)	0.52±0.04 (8)	0.56±0.09 (8)	0.05±0.01 (8)	0.55±0.30 (2)
	Spartina	435.2±129 (5)	42.7±2.0 (8)	0.67±0.04 (8)	0.41±0.08 (7)	0.15±0.04 (7)	0.35±0.08 (3)
	Halophytes	NA	42.4±1.5 (8)	0.81±0.03 (8)	0.44±0.11 (6)	0.33±0.11 (6)	0.13±0.08 (4)
Restored	Mudflat	NA	NA	NA	NA	NA	NA
	Creek-bank	NA	41.9±1.4 (8)	0.45±0.05 (8)	0.82±0.03 (8)	0.05±0.03 (8)	0.87±0.09 (4)
	Spartina	286.0±55 (8)	42.5±1.1 (8)	0.45±0.05 (8)	0.68±0.05 (8)	0.04±0.01 (8)	0.37±0.09 (5)
	Halophytes	NA	55.9±5.6 (8)	0.30±0.02 (8)	0.81±0.05 (6)	0.02±0.01 (6)	0.59±0.24 (2)
ANOVA	Habitat (C S X)	NA	**0.022** (a ab b)	0.100 (a a a)	**0.014** (a b ab)	**0.014** (a ab b)	**0.009** (a b b)
	Status	>0.2	0.06	**<0.0001**	**<0.0001**	**0.0001**	**0.010**
	Status x habitat	NA	**0.047**	**<0.0001**	>0.5	**0.0014**	>0.2
TIJUANA NAT	Mudflat	NA	55.5±4.9 (11)	0.38±0.04 (12)			
	Spartina	146.5±25.1 (11)	45.5±1.7 (10)	0.40±0.04 (12)			
REST	Mudflat	NA	60.6±2.0 (11)	0.54±0.03 (12)			
	Spartina	137.1±13.1 (11)	82.1±5.5 (10)	0.55±0.03 (12)			
ANOVA	Habitat (M S)	NA	>0.3	>0.6			
	Status	>0.8	**<0.0001**	**<0.0001**			
	Status x habitat	NA	**0.0002**	>0.8			

material ($F_{1,37}=20.9$, p=0.0001). The greatest natural-restored differences in water

and organic matter content occurred in MIX sediments where restored sediments had

much less water (81% versus 30%) and substantially less organic matter (38% versus

2%). Restored wetland sediments were sandier than natural wetland ($F_{1,37}=41.0$,

p<0.0001). Sediment sand and organic matter also differed at the habitat scale (both

$F_{2,42}=4.8$, p=0.014). Light transmission to the sediment surface was higher in the

restoration site ($F_{1,12}=8.7$, p=0.010) and also was generally higher in unvegetated

(CRK and MUD) areas than in vegetated sediments (habitat level comparison:

$F_{2,12}=6.6$, p=0.009). Within *Spartina* marsh, there was no difference between natural

and restored *Spartina* shoot densities ($F_{1,11}=1.5$, p=0.25; Table 2.2).

At Tijuana Estuary, restored marsh sediments had higher salinities than

natural marsh habitat ($F_{1,38}=35.8$, p<0.0001), but a highly significant interaction term

($F_{1,38}=17.0$, p=0.0002) and lack of a habitat-level effect ($F_{1,38}=1.1$, p>0.3) suggest that

real differences only existed between the natural and restored *Spartina* salt marsh.

The moisture content of sediments was higher in natural habitat than restored

wetland ($F_{1,44}=21.1$, p<0.0001) but did not differ between MUD and SPAR ($F_{1,44}=0.2$,

p>0.6). Like Mission Bay, *Spartina* densities were similar in restored and natural

wetlands ($F_{1,20}<0.1$; p>0.8; one-factor ANOVA).

Microphytobenthic abundance. Mean chlorophyll a concentrations in

surface sediments were significantly higher in natural Mission Bay sediments than in

restored habitat ($F_{1,42}=9.6$, p=0.003; Figure 2.2). No chlorophyll a differences existed

across habitats ($F_{2,42}=3.0$, p=0.06) but a significant habitat-status interaction

($F_{2,42}=5.7$, p=0.006) suggests that the magnitude of restored-natural differences was

habitat-specific. Sediment bacteriochlorophyll a concentrations were 1-2 orders of

magnitude smaller than chlorophyll a abundances. In contrast to chlorophyll a,
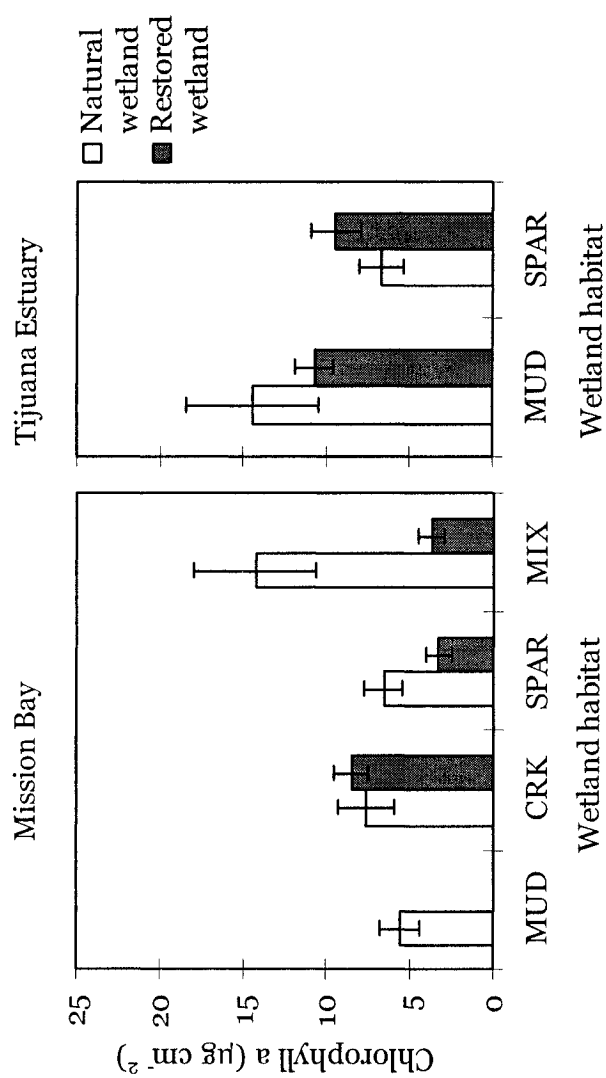
Figure 2.2. Spatial variation in mean (±1 S.E.) chlorophyll a concentrations (oxygenic phototroph biomass) in sediment from Mission Bay (left) and Tijuana Estuary (right). Habitats are found, generally, at increasing tidal elevation from left to right within each site. MUD=mudflat, CRK=creek-bank, SPAR=*Spartina* salt marsh and MIX=mixed halophyte marsh.
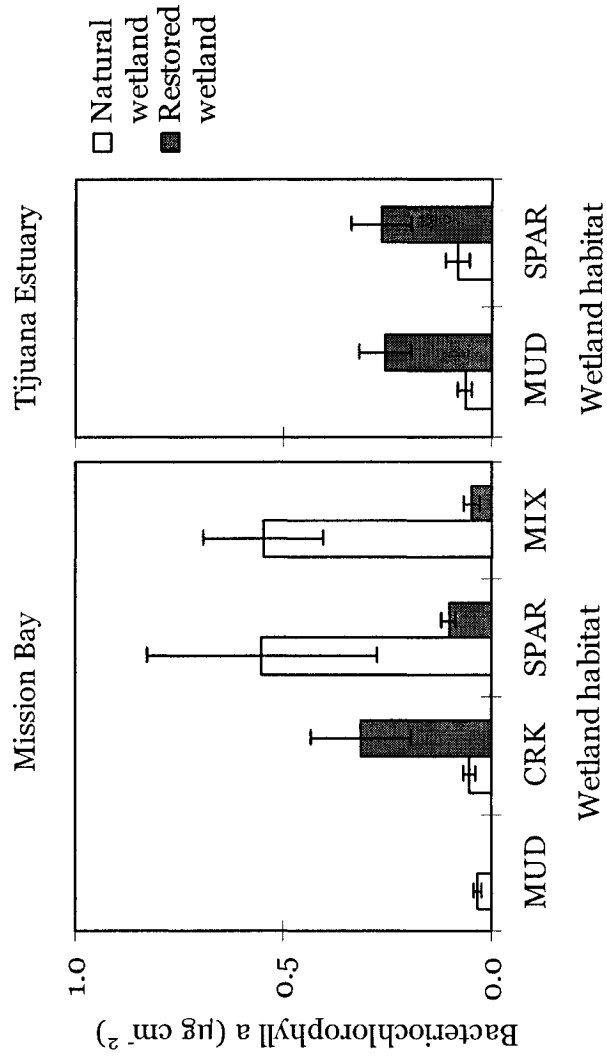
Figure 2.3. Spatial variability in mean (±1 S.E.) bacteriochlorophyll a concentration (anoxygenic phototroph biomass) in Mission Bay (left) and Tijuana Estuary (right) sediments.

bacteriochlorophyll a abundances were similar between natural and restored wetland sediments ($F_{1,42}$=3.4, p=0.07; Figure 2.3). There was no overall habitat-level difference in bacteriochlorophyll a ($F_{2,42}$=0.5, p>0.5), but because natural vegetated marshes (SPAR and MIX habitats) showed markedly higher levels of bchl a than restored sediments, and the direction of the natural-restored disparity was reversed in creek-banks (where ~6 times more bacteriochlorophyll a was present in restored channels than in natural creeks), there was a highly significant status-habitat interaction ($F_{2,42}$=7.7, p=0.001).

Chlorophyll a abundances at Tijuana Estuary were similar between mudflat and *Spartina* marsh habitat ($F_{1,44}$=1.8, p=0.18), and also showed no differences due to restoration status ($F_{1,44}$=1.7, p>0.2; Figure 2.2). Bacteriochlorophyll a concentrations, however, were approximately 3-4 times higher in both restored MUD and SPAR habitats (0.25 and 0.26 µg cm^{-2} respectively) than in natural MUD and SPAR sediments (0.06 and 0.08 µg cm^{-2} respectively; $F_{1,22}$=17.0, p=0.0004; Figure 2.3). No significant habitat-level difference in bacteriochlorophyll a was evident ($F_{1,22}$=0.8, p>0.3) at this site.

Composition and diversity. Bchl a/chl a ratios were similar between habitats ($F_{2,42}$=2.5, p=0.093) and between restored and natural wetland ($F_{1,42}$=0.4, p>0.5) in Mission Bay, but there was a significant habitat-status interaction ($F_{2,42}$=3.5, p=0.04). In halophyte-dominated salt marsh, bchl a/chl a of natural habitat exceeded that of restored habitat by nearly 5 times ($F_{1,13}$=5.0, p=0.04, one-factor ANOVA; Figure 2.4). Zeax/fuco ratios differed across habitats in Mission Bay ($F_{2,42}$=3.3, p=0.047) where vegetated habitats from both restored and natural wetland areas in Mission Bay generally showed about twice as much zeaxanthin relative to fucoxanthin than sediments from unvegetated mudflat and creekbanks (Figure 2.5).
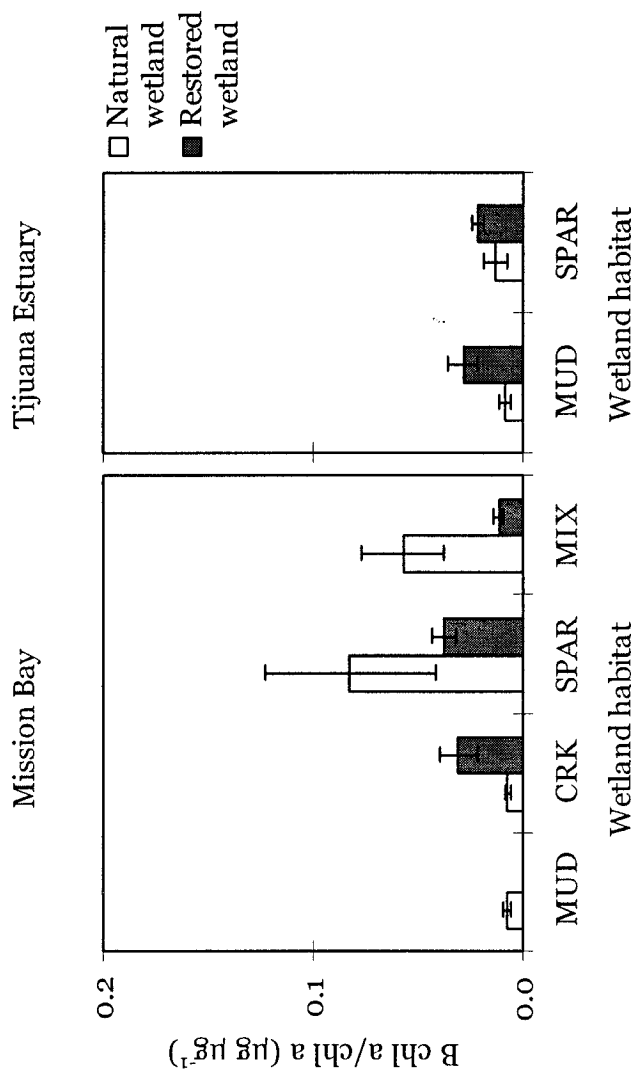
Figure 2.4. Habitat-level and restored-natural wetland differences in the mean (±1 S.E.) concentration of bacteriochlorophyll a relative to chlorophyll a (anoxygenic phototroph versus oxygenic phototroph biomass) in Mission Bay (left) and Tijuana Estuary (right) sediments.
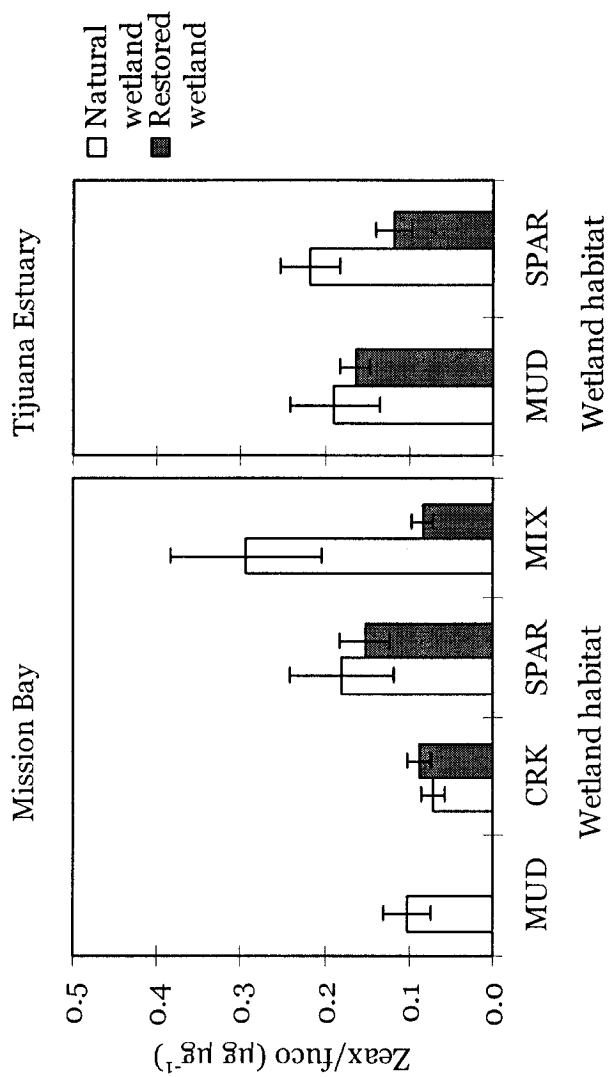
Figure 2.5. Mean (±1 S.E.) abundance of zeaxanthin relative to fucoxanthin (cyanobacteria versus diatoms) in sediments from different habitats inand restored and natural wetlands in Mission Bay (left) and Tijuana Estuary (right).

There was only marginal evidence for overall zeax/fuco differences due to marsh status ($F_{1,42}=3.1$, p=0.084). However, zeax/fuco was higher in natural wetland than restored habitat when mixed halophyte salt marsh was considered alone ($F_{1,14}=6.1$, p=0.027, one-factor ANOVA).

Functional diversity in Mission Bay sediments differed across habitats ($F_{2,42}=3.5$, p=0.04; Figure 2.6). The highest functional group diversities were observed in the both restored and natural SPAR marsh (restored= 1.65±0.15; natural= 1.83±0.26) and in MIX salt marsh from the natural wetland (1.96±0.23). At the wetland status scale, there was evidence of higher functional diversity in natural habitat ($F_{1,42}=4.2$, p=0.05). Moreover, a significant habitat-status interaction ($F_{2,42}=3.5$, p=0.04) was likely due to the higher diversity of microproducers in halophyte communities in natural wetland relative to restored marsh ($F_{1,13}=8.8$, p=0.01, one-factor ANOVA).

At Tijuana Estuary, bchl a/chl a exhibited no differences due to habitat ($F_{1,22}=1.3$, p>0.2), but strongly differed between restored and natural wetland ($F_{1,22}=14.8$, p=0.0009). Zeax/fuco was likewise similar between mudflat and *Spartina* habitats ($F_{1,41}<0.1$, p>0.9) whereas natural wetland was similar to that of restored habitat ($F_{1,41}=1.0$, p>0.3). Finally, functional group diversity was similar across habitats and between natural and restored wetlands (both $F_{1,22}<0.2$, p>0.7).

In pooled microphytobenthic communities from Mission Bay (n=56), multivariate analyses of pigment composition suggested little difference between habitats (p=0.09, ANOSIM) or between restored and natural wetlands (p>0.4, ANOSIM; Figure 2.7a). Likewise, there was no difference in the microphytobenthic composition of Tijuana Estuary communities (n=26) across habitats (p>0.9) or according to wetland status (p>0.2, ANOSIM; Figure 2.7b). Although restored
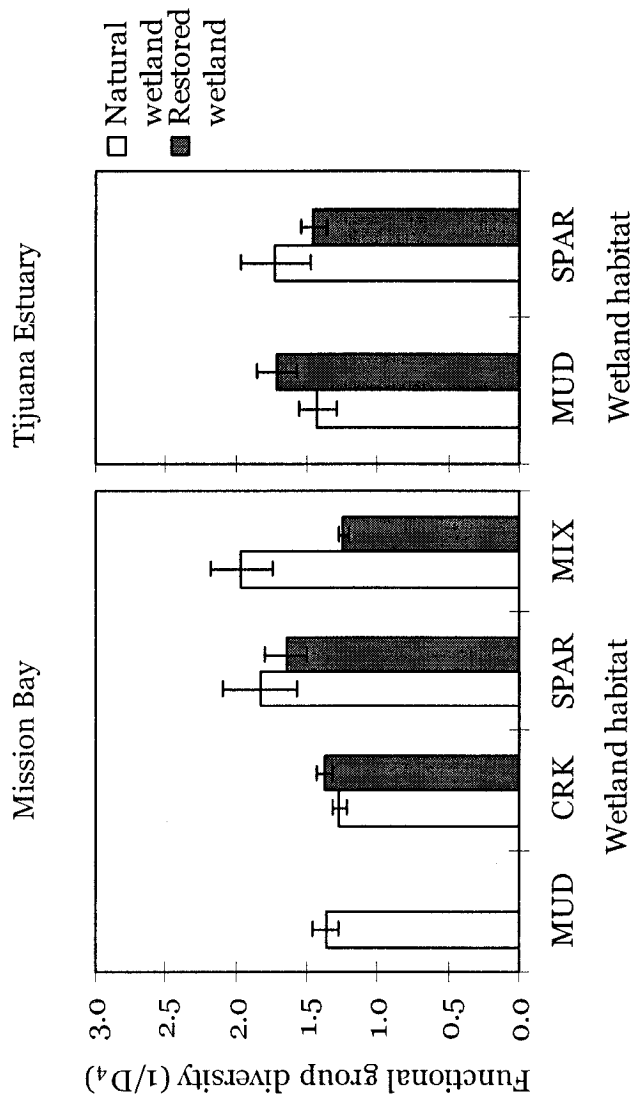
Figure 2.6. Spatial heterogeneity in the mean (±1 S.E.) diversity of microphytobenthic taxonomic (functional) groups in Mission Bay (left) and Tijuana Estuary (right) wetland sediments. Simpson's diversity index (1/D_4) was calculated based on the abundances (in µg cm⁻²) of lutein (from green algae and plant detritus), fucoxanthin (diatoms), zeaxanthin (cyanobacteria), and bacteriochlorophyll a (anoxygenic phototrophic bacteria).

sediment community composition at both sites clearly fell within the two-dimensional space occupied by natural wetland assemblages (see Figures 2.7a, b), it was also evident that restored communities largely did not attain the same breadth of compositional heterogeneity that characterized natural wetlands. A site-level comparison of functional group composition suggested minor differences between Mission Bay and Tijuana Estuary assemblages (p=0.012, ANOSIM; Table 2.2). This difference appeared to be driven by higher dominance of bacteriochlorophyll a (anoxygenic phototrophic bacteria) and zeaxanthin (cyanobacteria) in Mission Bay and higher dominance of lutein (green algae/plant detritus) and fucoxanthin (diatoms) in Tijuana Estuary.

Virtually all pigment assemblages at both sites, regardless of habitat type or restoration status were heavily dominated by fucoxanthin (from diatoms), but based on raw pigment abundances alone, bacteriochlorophyll a, zeaxanthin and/or lutein concentrations occasionally comprised more than 15% of the total taxon-specific pigments (B_4) present in a community (Figure 2.8).

Sediment environment and microphototroph community composition. In natural wetland communities from Mission Bay, there was no significant relationship between pigment measures of composition and diversity and sediment salinity, sediment water content, the proportion of sand sized-particles, or light transmission to the sediment surface (all p>0.05). However, the proportion of organic matter in surface sediments was positively related to the ratios of bacteriochlorophyll a to chlorophyll a (ρ=0.61, p=0.0005) and zeaxanthin/fucoxanthin (r=-0.37, p=0.049). Organic matter was also positively associated with functional group diversity within natural assemblages (ρ=0.58, p=0.001). At Mission Bay's restored wetland site, measures of pigment composition
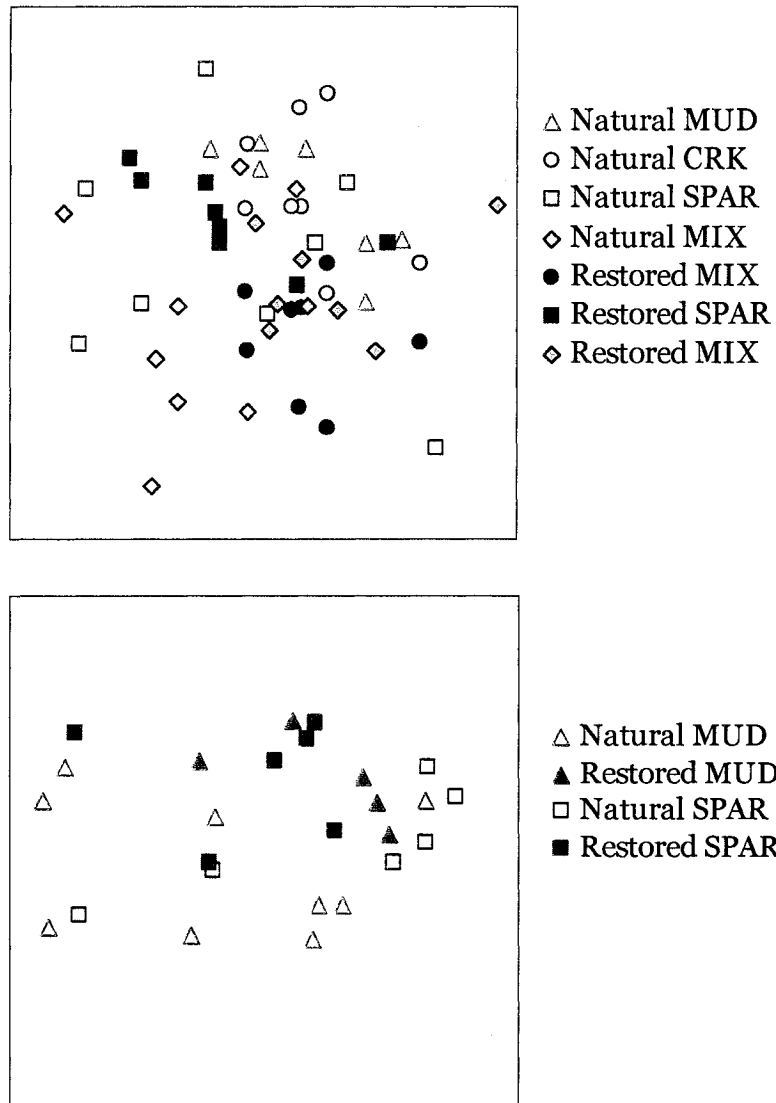
Figure 2.7. Non-metric multidimensional scaling (nMDS) representation of spatial differences in the composition of microproducer communities from (a) Mission Bay (n=56) and (b) Tijuana Estuary (n=26) sediments during spring 2002. These two dimensional representations are based upon a similarity matrix generated from the abundances (4th-root transformed dominance) of fuco, lute, zeax, and bchl a. Normalization of each pigment to total diagnostic pigment abundance (B_4) eliminated the effects of variable community biomass. Spatial proximity is proportional to compositional similarity. Stress= 0.10 for Mission Bay, 0.03 for Tijuana Estuary.

Table 2.3. Multivariate comparisons of microphytobenthic community composition according to habitat, restoration status, and site. Functional group composition was characterized by the abundances of four taxon-specific pigments: fucoxanthin (diatoms), lutein (green algae/plants), zeaxanthin, (cyanobacteria), and bacteriochlorophyll a (anoxygenic photo-trophs) each normalized to the total abundance of diagnostic pigments to eliminate differences due to variability in community biomass. The slight Mission Bay-Tijuana Estuary difference in composition was driven by lutein (35%), bacteriochlorphyll a (32%), zeaxanthin (23%), and fucoxanthin (10%) dominances respectively (SIMPER).

	Comparison	n	SIMPER Within-group SIM	Between-group DISSIM	ANOSIM p
within Tijuana Estuary	Spartina marsh	12	89.6%	10.3%	>0.9
	(vs) mudflat	14	88.7%		
	Natural (vs)	15	87.5%	10.5%	>0.2
	Restored wetland	11	92.7%		
within Mission Bay	mudflat	8	92.3%	from 7.3% to 10.4%	0.087
	creek-banks	16	93.0%		
	Spartina	16	89.5%		
	mixed halophytes	16	90.0%		
	Natural (vs)	32	89.9%	9.2%	>0.4
	Restored wetland	24	92.1%		
Between Sites	Mission Bay (vs)	24	89.8%	11.2%	**0.012**
	Tijuana Estuary	26	89.4%		
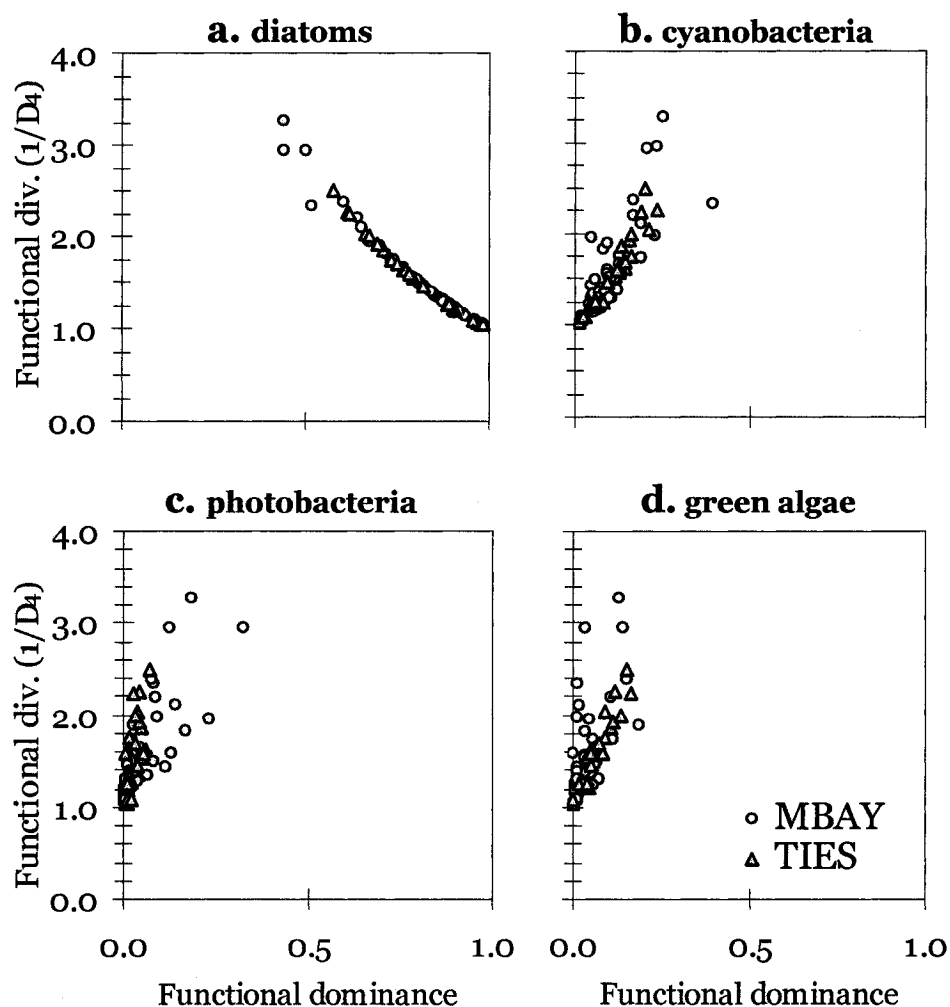
Figure 2.8. Relationships between functional group diversity and the dominance of each major functional group in pooled sediment assemblages from Tijuana Estuary (TIES; n=26) and Mission Bay (MBAY; n=56). (a) Diatom dominance (fuco/B_4) versus functional diversity (1/D_4), (b) cyanobacterial dominance (zeax/B_4), (c) phototrophic bacterial dominance (bchl a/B_4), and (d) green algal (lute/B_4) dominance. Maximum diversity (at 1/D_4=4) would be found in a community of highest evenness of all four functional groups (when p_p=0.25 for each taxon).

and diversity were not associated with sediment pore water retention, salinity, light transmission, or particle size distribution (each p>0.1), but organic matter was correlated with bchl a/chl a (ρ=0.62, p=0.002) and functional diversity (ρ=0.46, p=0.030). No relationship existed between organic matter and zeax/fuco in the restored wetland (p>0.4).

Discussion

Measurements of the abiotic conditions associated with sediments and overlying plant canopies at these two southern California sites suggest that certain features of the sediment environment vary both between habitats and between restored and natural wetlands. At Mission Bay, obvious differences in light regimes were observed at the habitat-level but differences in organic matter and sediment sand, and salinity were also seen. Interestingly there was no variation in sediment moisture or salinity between SPAR salt marsh and mudflats in Tijuana Estuary. Natural and restored wetland microenvironments were also significantly different for many of the abiotic features (e.g., sand, organic matter) considered in this study. In general, restored marshes were more saline, had more open plant canopies (greater light transmission), more sand and less organic matter than nearby natural habitat. However, the magnitude and direction of these differences were often dependent upon the landscape or site under consideration. For example, the water content of sediments in Mission Bay was greater in natural vegetated landscapes (SPAR, MIX) relative to restored salt marsh habitat, but creek-bank sediments maintained about the same amount of moisture (45-52%) during low tide. At Tijuana Estuary, restored wetland sediments held more water but the opposite was true in Mission Bay.

Of all habitats considered in Mission Bay, mixed halophyte marsh (MIX) showed the most extreme differences between restored and natural conditions for virtually all abiotic variables measured. Earlier work at this site by Levin and Talley (2002) in the late 1990's comparing natural *Spartina* marsh with restored *Spartina*+other halophyte marsh showed that this small restoration site had substantially lower cover of vascular plants, higher salinities, less organic matter and more sand relative to natural wetland. The comparisons here (Table 2.2) suggest that this restored-natural disparity in the sediment environment persisted into spring 2002.

Differences in pigment composition largely paralleled the results from abiotic comparisons. For example, consideration of pigments from just natural wetland in Mission Bay suggests distinct habitat-level differences in microphototroph biomass, composition, and functional diversity between vegetated (SPAR and MIX) and unvegetated (CRK and MUD) environments (Figures 2.2-2.6), much like that seen in organic matter, light, and water content. Vegetated habitats tended to be characterized by higher taxonomic diversity, more bacteriochlorophyll a, and an increased proportion of zeax/fuco (cyanobacteria versus diatom biomass). In a comparison of estuarine pigments, Brotas and Plante-Cuny (1998) also noted higher zeaxanthin concentrations in salt marsh than in mudflat, but it is unknown if this could be merely reflective of differences in light regimes.

Reduced light flux and/or higher organic matter at the sediment surface due to the vascular plant canopies in salt marshes may favor cyanobacteria and phototrophic bacteria over diatoms. Several significant correlations between composition (zeax/fuco and bchl a/chl a) and sediment organic matter suggest that this feature of the sediment environment may enhance the abundance of prokaryotic

producers in salt marsh relative to mudflats. Despite its obvious importance to microproducers, light effects on the relative abundance of benthic cyanobacteria are inconclusive. In the manipulative work of Sullivan (1976) at a Delaware salt marsh, cyanobacteria appeared to respond favorably to higher irradiance (via canopy removal), but in a similar experiment at a site in Mississippi, no cyanobacterial "bloom" was observed (Sullivan 1981). The Mission Bay pigment concentrations reported here suggest an enhancement of cyanobacterial dominance under plant canopies, but no effect at Tijuana Estuary (Figure 2.5). Further manipulative experimentation on the role of light, organic matter, and other sediment factors is needed to understand how the abiotic environment directly controls the functional group composition and diversity of microphototroph communities. Regardless of the direct cause of shifts in composition, greater functional diversity was probably observed in vegetated salt marsh relative to unvegetated wetland because these prokaryotic components of the microflora were more abundant in vegetated habitats (Figures 2.3, 2.5, 2.6, 2.8).

In contrast to Mission Bay, habitat-level variation in pigment communities at Tijuana Estuary was absent. *Spartina* salt marsh and mudflat had similar chlorophyll a and bacteriochlorophyll a concentrations, functional diversity, and zeaxanthin/fucoxanthin ratios (Figures 2.2, 2.3, 2.5, 2.6). Salt marsh and mudflat also showed similar water content and sediment salinities. Lack of any habitat-level variability in both the abiotic and biological variables measured suggests a surprisingly high similarity between *Spartina* habitat and mudflat surface sediment despite the obvious difference in light regimes.

Sediment pigment concentrations also suggested the presence of microproducer community differences between restored and natural wetlands. At

Mission Bay these differences were evident only for measures of phototroph biomass (chlorophyll a and bacteriochlorophyll a concentrations; Figures 2.2, 2.3). Composition and functional diversity differences at this site due to restoration status were limited to the mixed halophyte salt marsh and were absent from *Spartina*-dominated salt marsh and creek-bank habitat. Tijuana Estuary, which did not exhibit habitat-level variation in microproducer communities, also largely failed to show substantial natural-restored wetland differences. Only bacteriochlorophyll a concentrations differed between restored and natural wetland (Figure 2.3). This apparently rapid recovery of microphytobenthic community diversity (by at least 26 mo) at Tijuana Estuary parallels the observations on metazoan assemblages made by Moseman et al. (2004) who found similar diversity in natural and restored wetland at 19 months of succession. No microphytobenthic communities from halophyte salt marsh or intertidal channel habitats were studied at Tijuana Estuary and restored-natural differences in microproducer assemblages might have existed within other kinds of habitats.

Natural versus restored differences in microphytobenthic community composition may be driven partially by pronounced differences in the sediment environmental characteristics of restoration sites. For example, much lower organic matter at the restored site in Mission Bay (Levin and Talley 2002; Table 2.2) may lead to lower functional group diversity and a shift toward eukaryotic producers over bacterial carbon fixers. Lower organic matter in restored wetlands (even after a few decades) appears to be a common feature of marine wetland rehabilitation efforts (Craft et al. 1988, Moy and Levin 1991, Talley and Levin 1999, Edwards and Proffitt 2003). Measurements at Mission Bay also showed that sediments were sandier in restored wetland (Table 2.2), with possible effects on sediment water retention at low

tide and light penetration. Hydrologic differences may also exist between natural and restored habitats. Restored wetlands at both sites are (a) connected to natural habitat via a single channel and (b) possess an artificial channel network that may not adequately reflect natural drainage. For example, the higher bacteriochlorophyll a concentrations in restored creeks in Mission Bay and in both landscapes in Tijuana Estuary relative to comparable natural habitat (Figure 2.3) may be due at least partially to poorer sediment oxygenation linked to larger-scale hydrology.

Throughout this study, the scale of variation for any given measure of microphytobenthic assemblages appeared to be dependent upon the site under consideration. In particular, the environmental differences (or lack thereof) observed at the landscape and wetland status scales at Mission Bay and Tijuana Estuary largely paralleled the variation observed in microphytobenthic communities. At Mission Bay, variation across landscapes appeared to be a more consistent feature of microphytobenthic community composition and diversity than variation due to the status of wetland habitat, whereas the single detected difference among communities at Tijuana Estuary (bacteriochlorophyll a) was found between natural and restored wetland. These scale-associated differences between the two sites considered could reflect any of a suite of unique site-based features including wetland habitat size and differences in the kinds and frequency of disturbance. Measures of microphototroph communities in the restored wetland at Tijuana Estuary (age: 2.2 yr) suggested full microphytobenthic recovery except for a two-fold difference in bacteriochlorophyll a (Figure 2.3). The more mature restoration site at Mission Bay (age: 6.3 yr) suggested recovery of *Spartina* salt marsh but lack of similarity between creek-banks and halophyte salt marsh. In particular, restored halophyte marsh consistently failed to resemble natural wetland in virtually all investigated measures of microproducer

assemblages.

At both sites examined in this study, the same major functional groups tend to be present in communities of sediment microphototrophs, suggesting that diversity differences between assemblages were due principally to changes in constituent evenness. For the sites and period studied, the basic microphytobenthic assemblage appears to have been dominated by diatoms, only occasionally punctuated by the occurrence of other major functional groups in substantial abundance. The preponderance of diatoms in the microalgal assemblages of most shallow coastal sedimentary habitats has been observed in disparate geographical regions (Sullivan and Moncreiff 1988, Underwood 1994). Thus, localized sites of higher functional diversity (perhaps occurring largely on spatial scales smaller than those investigated here; see Pinckney and Sandulli 1990) may coincide with spatial patches where ecosystem processes mediated by prokaryotic phototrophs (nitrogen fixation, sulfide oxidation, anoxygenic photosynthesis) are enhanced.

Conclusions. Overall, the pigment-based research presented here shows spatial patterns with several important ecological and management implications. First, among the spatial scales tested, habitat-level differences (seen in anoxygenic phototroph biomass, functional group composition, and functional diversity) appeared to be the most important level of variation within Mission Bay, whereas very little evidence of differences due to either marsh status or habitat was evident at Tijuana Estuary. These qualitative site differences in community variability may be reflective of site-specific disturbance histories, wetland size, or other spatial differences and suggest that site-specific attributes need to be considered in restoration work.

Second, the mixed halophyte salt marsh at Mission Bay was distinctly different

between natural and restored wetland for both abiotic measures of the sediment environment and for measures of microproducer biomass, composition, and diversity, suggesting the need to carefully evaluate the biotic composition of all landscapes present in wetland restoration efforts to ensure their equivalency with natural habitat. Because mixed halophyte marsh is very common at Mission Bay on an aerial basis, future restoration work needs to ensure recovery of this particular landscape is met to support ecosystem-wide functions. Recovery of these high organic matter habitats may take much longer than corresponding *Spartina* salt marsh or unvegetated mudflat.

Finally, the simple existence of distinct spatial heterogeneity in wetland microproducer composition and diversity at the functional group (higher taxonomic) level suggests the desirability of determining if heterogeneity in important ecosystem functions such as primary production and nutrient cycling is also coupled to the spatial differences in microphytobenthic communities. Localized sites (of whatever scale) where communities are shifted more towards cyanobacteria and anoxygenic phototrophs may be locations of increased sulfide oxidation, more nitrogen fixation, and more efficient carbon utilization because of the possible existence of photo-heterotroph and anoxygenic-oxygenic phototroph consortia (Paerl et al. 1993, Pinckney and Paerl 1997). To further our understanding of these critical dynamics and more fully evaluate restoration efforts, it is necessary for wetland ecologists to move beyond aggregate measures of microproducer communities (e.g., chlorophyll a) to better understand processes mediated by the microflora.

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Chapter III.

Succession of microalgae and phototrophic bacteria in a restored marine wetland:

a functional group perspective

Abstract

Mitigation for the degradation of coastal marine wetlands is often accomplished by creation of novel habitat or restoration of land formerly inundated by tides – conservation efforts that also become large-scale experiments in disturbance and the succession of biological communities. The longer-term (>1 yr) succession of benthic microproducers (diatoms, cyanobacteria, green algae, phototrophic bacteria), which are functionally important to many marine wetland ecosystems, is virtually unknown in restoration work. Using taxon-specific photopigments, I describe the composition of restored microphytobenthic communities and investigated differences in the temporal recovery of biomass (chlorophyll a), composition, and functional diversity between vegetated *Spartina* salt marsh and unvegetated mudflat in a large restoration project in southern California. Visually distinct, spatially discreet microphytobenthic patches appeared after no more than 7 mo within the restoration site and were distinguished by significant differences in biomass (chlorophyll a), functional group diversity, and the relative abundance of cyanobacteria versus diatoms. Overall, microphytobenthic biomass (chlorophyll a) within the restored site was comparable to natural biomass across most sampling dates suggesting rapid colonization by microproducers. Additionally, restored *Spartina foliosa* habitat very rapidly (between 0.2 and 1.2 yr) acquired microphytobenthic communities of similar functional group composition and diversity to those in natural *Spartina* habitat, but restored mudflats took at least 1.5-2.2 yr to resemble natural mudflats. These results suggest relatively rapid structural equivalency of microphytobenthic communities at the functional group level. The data also imply that provision of structural heterogeneity (such as pools and vascular

plants) in wetland construction might speed development of microfloras, but no direct "seeding" of sediment microfloras may be necessary.

Introduction

Wetland microproducers. Sediment-dwelling microproducers such as diatoms, cyanobacteria, green algae, and phototrophic bacteria are of central importance to marine wetland food webs and to the functioning of coastal sedimentary habitats. Primary production by benthic algae and phototrophic bacteria (also collectively called "microphytobenthos" or 'microproducers') can rival the contribution made by vascular plants to ecosystem production (Zedler 1980, Fejes et al. 2005) and often ends up supporting wetland consumers such as benthic macrofauna, meiofauna, and fishes (Kwak and Zedler 1997, Page 1997, Buffan-Dubau and Carman 2000). Wetland microproducers also function to stabilize sediments (Gust and Grant 1987, Austen et al. 1999) and to mediate the fluxes of nitrogen and other nutrients through coastal habitats (Tyler et al. 2003).

Much of the research on the temporal variability of wetland microphytobenthic communities has focused on short-term or seasonal changes in assemblage abundance. For example, work by Underwood and Paterson (1993) showed seasonal differences in sediment chlorophyll a. Community composition may also change seasonally: Currin and Paerl (1998), for example, found shifts in cyanobacterial taxa resident on dead *Spartina* shoots. Several researchers have found that winters tend to be characterized by higher diatom and green algal densities, whereas cyanobacteria and euglenoids are more common in summer floras (Carter 1933, Sage and Sullivan 1978, Zedler 1982). Using photopigment concentrations to track abundances of major taxonomic groups, Pinckney et al. (1995) noted shifts in

the relative proportion of diatoms and cyanobacteria and over a year's sampling in a North Carolina estuary, again finding that cyanobacteria were more dominant in summer months.

Although it is fairly well understood that various benthic microalgae in marine wetlands (particularly diatoms and cyanobacteria) experience seasonal fluctuations in abundance, few studies have been conducted on longer-term changes in community abundance, composition, or biodiversity. Stal et al. (1985) noted a shift from an *Oscillatoria* (=*Lyngbya*?)-dominated to *Microcoleus*-dominated community over approximately two years at an intertidal sand flat in the North Sea. Peletier (1996) examined changes in a mudflat diatom flora following reduced eutrophication and found a decadal-scale shift in the dominant *Navicula* species. Anecdotal evidence suggests a long-term rise in green algal blooms in Elkhorn Slough in central California, possibly a consequence of salt marsh recession (Zimmerman and Caffrey 2002). Much more research needs to be conducted on longer-term temporal variation in benthic microproducer assemblages and the factors (human and otherwise) that regulate such dynamics.

Wetland restoration. In many coastal regions worldwide, wetland restoration and rehabilitation attempts are underway to mitigate past and present destruction of intertidal habitats or to enhance stocks of selected taxa (Zedler 1996a). At three sites (among the larger of the coastal lagoons) in San Diego County in southern California for example, approximately 85% of all tidal wetlands have been destroyed during the last 200 yr (Zedler 1996b). In the typical restoration projects in the region, former tidal land (which was subsequently filled with soils) or upland areas adjacent to tidally-influenced marine habitat are mechanically graded down to intertidal heights to expand aerial coverage of habitat. Various engineering

experiments built into these restoration efforts (including the provision of intertidal channels and transplantation of native vegetation) are underway in the region to test mechanisms of colonization, tempo of recovery, and techniques for successful habitat rehabilitation (Zedler et al. 2001).

Only a few studies have addressed the question of microproducer succession in restoration work. Underwood (1997) documented three years of succession of wetland microproducer communities at a marsh in Britain and found compositional differences associated with different habitat types at the site. Several sites showed gradually declining chlorophyll a concentrations over several years (but no reference marsh comparison was conducted) and like Stal et al. (1985), he found a transition from *Oscillatoria* to *Microcoleus* succession at one low-intertidal location. Zheng et al. (2004) used replicate restored sites of variable age (1 to 25 yr) and paired natural sites across North Carolina to examine temporal trajectories in salt marsh microproducer recovery. Sediment chlorophyll a of restored wetlands appeared to have recovered quickly and the similarity between restored and natural functional groups was did not appear to be influenced by marsh age, but diatom species composition was shown to gradually become more similar between restored and natural habitat as the age of sites increased.

Restoration in southern California tidal wetlands. Tijuana Estuary is one of the largest extant tidal wetlands along the southern California coast and has been home to at least two wetland restoration projects. In 1997, a small (<1 hectare) wetland was constructed in the northeastern area of the estuary (Callaway et al. 2003). During 1999-2000, an additional site, the 8 hectare Friendship Marsh, was constructed south of the mouth of Tijuana Estuary. Here, a kidney bean shaped site of fine-grained sediments was designed containing six blocks with alternating tidal

creeks and mudflat (Figure 2.1). A 30 m swath of *Spartina foliosa* (Poaceae) was planted at several densities across the center strip of the six blocks and additional halophyte species were planted in upper intertidal locations (King et al. 2001, Zedler et al. 2001). Tidal influence commenced at the restoration site in February 2000 by linkage of a large restored marsh channel to natural wetland (Moseman et al. 2004).

I assessed the nature and tempo of microalgal and phototrophic bacterial succession at this restoration site by sampling one to three times annually for a period of three years in unvegetated mudflat and *Spartina foliosa* salt marsh. Using a pigment-based approach to the study of microphytobenthic composition and functional diversity, I addressed the following questions about succession in this sediment-based ecosystem: (1) Which major taxonomic groups were present in the restoration site during early succession? (2) What was the pigment composition of restored wetland sediment patches? (3) How long did it take for microphytobenthic abundance and composition to recover (i.e., to match the magnitude and variability in natural wetland) in mudflat versus *Spartina* marsh? (4) How did plant densities, sediment salinity and organic matter relate to microphytobenthic abundance, composition and diversity?, and (5) Were similar abiotic factors associated with variability in community structure in restored versus natural wetland?

Materials and methods

Field sampling. The 8 hectare Friendship Marsh was established in the southern portion of Tijuana Estuary by excavation of supra-littoral sediment fill that covered former intertidal habitat. A single, approximately 3 meter-wide, channel connects the restoration site to natural habitat; the rest of the wetland is separated

from tidal inundation by a soil berm. Three secondary creeks were connected with the main channel within the restored site (Zedler 1996a). During February 2000, following *Spartina* planting, a hydrologic connection was established with nearby natural habitat.

Sampling of benthic sediments was conducted during April 2000 (age=0.2 yr), September 2000 (0.6 yr), April 2001 (1.2 yr), June/July 2001 (1.4 yr), September 2001 (1.6 yr), April 2002 (2.2 yr) and April 2003 (3.2 yr) in restored and/or natural habitat (see Chapter II for additional analyses of April 2002 communities). For natural-restored wetland comparisons, (all April dates and September 2001), sediment collection and field observations were made within haphazardly-placed 0.25 m² quadrates situated inside six regions of *Spartina* marsh and nearby mudflat within the restored site and six patches of natural *S. foliosa*-dominated marsh and nearby mudflat in the southern arm of the estuary (Figure 3.1). Densities of all *Spartina* plants (alive or otherwise) ≥ approximately 10 cm in height were recorded. Percent cover of macroalgae (Chlorophyta) within each quadrat was visually estimated. One to three sediment cores for analysis of pigments by High Performance Liquid Chromatography (surface area of 0.57 or 0.95 cm²) were taken to 2-3 mm depth (April 2000), 5 mm depth (April 2002, 2003) or 10-20 mm depth (April 2001). Sediments were kept dark and later frozen (-20°C and/or -80°C) after field work was completed.

Several methods were used to study macroalgal and microproducer floras in the restoration site during early succession. To study flotation of macroalgae into the restored wetland, algal "traps" consisting of ~400 cm² wire mesh were suspended about 10 cm from the sediment surface with bamboo stakes in lower elevation mudflat during February 2000. After approximately two weeks, green macroalgae

were identified (Abbott and Hollenberg 1976). During September 2000 (restored

age=0.6 yr) and June-July 2001 (restored age=1.5 yr), sediment microproducers

within the restored wetland were also investigated by (a) microscopy and (b)

collection of sediment for pigment analyses from replicate patches of distinct

coloration and texture. In September 2000, collections of surface sediment (3 cores

of 2 mm depth per replicate) were made from within *S. foliosa* habitat and consisted

of the following target assemblages: LTH: yellowish, leathery patches, GRN: greenish
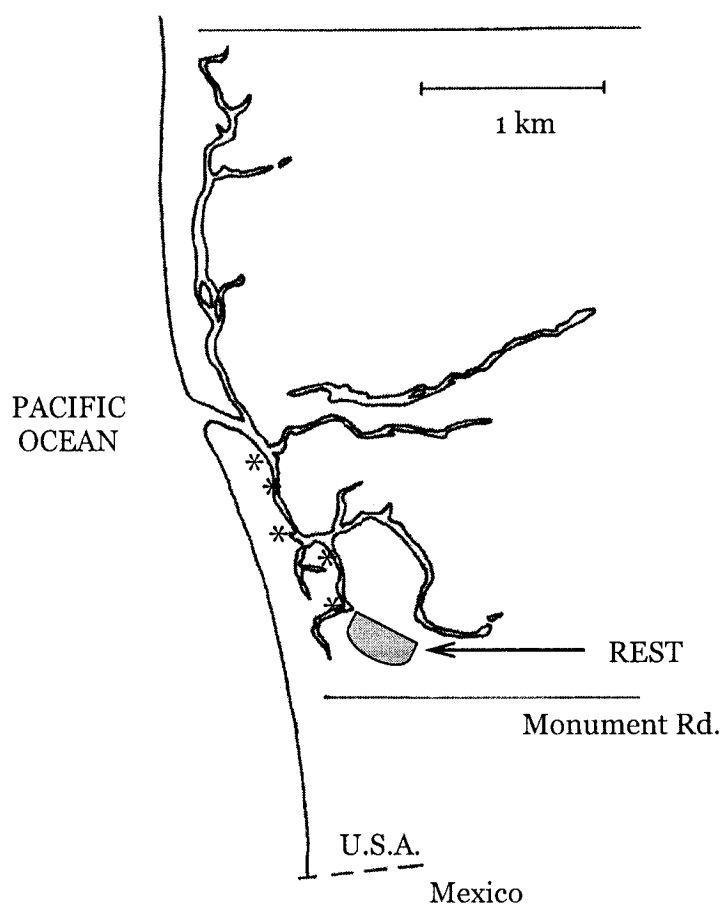
Figure 3.1. Tijuana Estuary National Estuarine Research Reserve, southern San Diego County, California. REST=restoration site The general areas from which natural wetland samples were collected are also shown (*). Natural wetland geographic features and roads after United States Department of Commerce (1981).

sediments, and CHL1: sediment from macroalgal (Chlorophyta)-dominated sediments.

In July 2001 sediment assemblages from *Spartina* marsh and upper (mostly) and lower elevation mudflat were sampled in the restored wetland. Three cores of sediment material were taken from 3-8 replicate patches of the following kinds of assemblages: "YEL," yellowish sediment frequently found in upper littoral pools, "GRN," deep-green-colored sediment in depressions and at edges of shallow pools, "CHL2," green macroalgae (Chlorophyta) and underlying sediment, "CHL3," sediment underlying macroalgae, "PNK," pinkish or pinkish-white (usually dry) sediment, and "BRN," sediment without distinct coloration. There was no attempt to synchronize the kinds of assemblages sampled during September 2000 and July 2001 (common patches were chosen during each date), although GRN patches may have been roughly equivalent in both periods. All sampling is summarized in Table 3.1.

Analyses of sediment pigments. Sediments were defrosted and treated with approximately 90% acetone, 10% seawater on ice for about 24 hr prior to separation via High Performance Liquid Chromatography (HPLC). To achieve a roughly similar composition of extraction solvent across samples, several techniques were used depending on sampling date. For the April 2000 and April 2001 work, estimates of sediment moisture for each habitat-site combination were made and used to determine the volume of acetone necessary to achieve extraction in 90% acetone. For the September 2001 to April 2003 samples, variation of sediment moisture in individual cores was determined by field collection of an extra sediment core adjacent to pigment cores. Moisture content was determined in the lab after weighing, drying (at ~60°C, ≥24 hr), and reweighing sediment and concentrations were then used to determine a per-core extraction volume of acetone. For the

Table 3.1. Sampling locations and dates in this study. Letters indicate the type of sampling: R= routine natural versus restored wetland comparison; P= patch-specific sampling; C= collection of macroalgal rafts. REST= restored wetland; NAT= natural wetland; MUD= mudflat; SPAR=*Spartina foliosa* marsh.

	REST			Sampling		
Date	age (yr)	REST MUD	REST SPAR	NAT MUD	NAT SPAR	
March 2000	0.1	C				
April 2000	0.2	R	R	R	R	
September 2000	0.7		P	R	R	
April 2001	1.2	R	R	R	R	
June-July 2000	1.4	P	P			
September 2001	1.7	R	R	R	R	
April 2002	2.2	R	R	R	R	
April 2003	3.2	R	R		R	

September 2000 samples, 3.0 ml of acetone was simply added to wet sediment regardless of initial water content.

Following extraction, sediment and supernatant were centrifuged and filtered through a cotton-fitted Pasteur pipette (Goericke 2002). Extracts were further diluted with water (5:12 water to solvent ratio) prior to analysis. Pigments were separated on one of two HPLC machines: (1) a Shimadzu SCL-10A System controller, Waters® 510 solvent pumps, and Waters® 991 Photodiode Array (PDA) or (2) a Waters® 600E pump and system controller coupled to a SpectraSYSTEM™ AS3000 autosampler, a Spectra 100 variable wavelength detector, and a Waters® 470 fluorescence detector. On system *1*, pigment abundances were determined by PDA absorption at 440 nm. On system *2*, pigments were detected by absorption at 450 nm and fluorescence detection (excitation at 430 nm, emission at 674 nm).

Several bi-phasic reverse-phase gradients were used for the separation of pigment extracts depending on the system utilized and as separation techniques evolved. September 2000 material was separated on a gradient ('A') developed by R. Goericke (SIO/UCSD) as follows: 0 min (100% phase 1/0% phase 2), 2 min (75%/25%), 30 min (50%/50%), 42 min (5%95%), 53 (5%/95%), and 45 min (100%/0%) where phase 1=methanol/0.5 M ammonium acetate/water/acetonitrile [30:10:30:30] and phase 2= methanol/ethyl acetate/acetonitrile [10:55:35]. April and June/July 2001 extracts were separated on a gradient similar to 'A' suitable for machine *2* (gradient 'C'). September 2001-April 2003 extracts were separated on an alteration of the bi-phasic gradient presented in Brotas and Plante-Cuny 1996 (gradient 'F'; see Appendix 2). Finally, several extracts from April 2002 were re-analyzed on machine *2* for determination of bacteriochlorophyll a concentrations using an alteration of the method of Goericke (2002) with absorbance detection at

700 nm (gradient 'G'; see Chapter II and Appendix 2). In gradients A, C, and F, extracts were separated on an Adsorbosphere® C_{18} HS 10 cm by 4.6 mm diameter column (3 µm stationary phase diameter); gradient G used an Adsorbosphere® C_{18} HS 15 cm by 4.6 mm diameter column (5 µm stationary phase).

Pure pigment standards obtained from DHI Water and Environment (Hoersholm, Denmark) and provided by M. Vernet (chlorophyll a) were used to calibrate system 2 (see Table A2.6). PDA calibration factors (at 440 nm) were provided by R. Goericke for system 1. Pigments were identified based on PDA-generated absorption spectra (~350-800 nm) and intermittent analysis of mono-specific plant and algal material (system 1) or by comparison of elution with plant and algal material (system 2). Extracts of *Thalassiosira weissflogii* and *Chaetoceros* (Bacillariophyceae), *Arthrospira platensis* (cyanobacteria), *Mentha* spp. (Lamiaceae, Anthophyta), *Dunaliella tertiolecta* (Chlorophyta), and *Lingulodinium polyedrum* (Dinophyta) served as standards (see Appendix 2 for pigment composition).

Sediment environmental variables. Sediment pore water salinities were determined on a refractometer in the field after crude filtration of sediment through filters fit inside 10 ml plastic syringes. Organic content was determined by mass loss in surface sediments (0-1 cm depth) after 550°C combustion of dry sediment for several hours (Carver 1971).

Estimation of community composition and diversity. The carotenoids fucoxanthin, zeaxanthin, and lutein were used to estimate the biomass of diatoms, cyanobacteria, and green algae/plant detritus respectively (Pinckney et al. 1995, Table 2.1). Bacteriochlorophyll a concentrations were used as an estimate of the biomass of anoxygenic phototrophic bacteria (mainly purple bacteria; Imhoff 1999) but were determined only for sediments collected in September 2000 and April 2002. Total

microphytobenthic biomass was estimated by either chlorophyll a or as the total sediment concentration of diagnostic carotenoids (B_3= fuco+lute+zeax) where fuco=fucoxanthin, lute=lutein and zeax=zeaxanthin concentrations (in μg cm^{-2}). Functional group composition was characterized by the ratio of zeaxanthin to fucoxanthin (μg μg^{-1}; Pinckney et al. 1995) or, for multivariate work, by determination of the individual dominance levels of each functional (i.e., for diatom dominance = fuco/B_3) to remove any variation in communities due to differences in total biomass (Chapter II). Functional group diversity was also determined for communities using Simpson's index ($1/D_3$= $[\Sigma\ p_p{}^2]^{-1}$ where p_p = fuco/B_3, lute/B_3 or zeax/B_3; Magurran 2004). Additional pigment concentrations (e.g., diatoxanthin, diadinoxanthin, chlorophylls b, $c_{1,2,3}$ and carotenes were determined via HPLC but inclusion of these into a diversity index or into multivariate compositional analyses would add redundancy and inflate true community differences.

Statistical tests. To test for spatial differences in the community composition of microproducers between different communities in the restored wetland (September 2001 and July 2001), one factor ANOVAs were performed on community biomass (chlorophyll a), the relative abundance of cyanobacteria versus diatoms (zeax/fuco) and functional group diversity ($1/D_3$). Initial tests and *a posteriori* Tukey-Kramer comparisons were performed at α=0.05, but significance levels of $0.1 > p > 0.05$ were considered marginally significant in cases of low replication.

Analysis of natural and restored wetland differences (chlorophyll a, zeax/fuco, $1/D_3$) was carried out for each successional time point (April 2000, April 2001, September 2001, April 2002 and April 2003) by one factor ANOVAs conducted separately in *Spartina* salt marsh and unvegetated mudflat. Independence of samples

across time was assumed because of (a) the relatively long periods (5-7 mo) between sampling points and (b) variation in exact sampling points from period to period.

Compositional differences between restored and natural wetlands were also investigated by non-metric multidimensional scaling (nMDS) based on a Bray-Curtis similarity matrix (generated from 4th-root transformation of pigment dominances: $fuco/B_3$, $lute/B_3$, and $zeax/B_3$). Heterogeneity between restored and natural mudflat and between restored and natural *Spartina* marsh assemblage composition was subsequently tested with ANOSIM (Primer 5 software, > 400 bootstraps). Within treatment homogeneity (e.g., similarity within restored *Spartina*) and between treatment heterogeneity (e.g., dissimilarity between restored and natural *Spartina*) were determined by SIMPER (Primer 5 software).

Finally, I investigated the influence of (a) sediment pore water salinity, (b) sediment moisture, (c) sediment organic content, and (d) *Spartina foliosa* densities on microphytobenthic abundance (chlorophyll a), composition (zeax/fuco), and functional diversity $(1/D_3)$ via single regressions. In each of these analyses, microphytobenthic assemblage data from April 2001, September 2001, April 2002, and April 2003 were pooled but separate analyses were conducted on each habitat-status combination (4 total). The normality of all variables used in ANOVAs and regressions were tested and data were transformed as needed (usually $\log_e$ or square root) prior to statistical tests. In a few rare cases, transformed distributions still showed substantial deviation from normality so Kruskall-Wallis or rank correlations (ρ) tests were used.

Results

Microproducer composition within restored wetland. Rafted macroalgae collected in early March 2000 (restored age ~0.1 yr) were assigned to *Ulva* spp., including tentatively *U. prolifera* O.F. Müller and *U. clathrata* (Roth) C. Agardh (see Hayden and Waaland [2004] for nomenclature). In September 2000 (restored age= 0.6 yr), various cyanobacteria (including *Oscillatoria* spp., *Microcoleus* and a heterocyst-bearing filament) and diatoms (*Cylindrotheca*, sigmoid and naviculoid pennate diatoms) were obtained from wetland sediments. Numerous algal and bacterial chlorophylls and carotenoids were obtained from both natural and restored wetland sediments at this time (Table 3.2).

By September 2000, microphytobenthic patches of distinct coloration and texture were also evident on the surface of sediments. In the three major assemblages sampled, sediment chlorophyll a differed between assemblages ($F_{2,9}$=14.8, p=0.001), with higher concentrations in LTH (yellowish, leathery sediments) and GRN (greenish sediment) patches relative to benthic phototroph communities in macroalgal-dominated (CHL1) sediments (Figure 3.2). Bacteriochlorophyll a abundances likewise differed across patches ($F_{2,9}$=5.0, p=0.04) and were significantly different between CHL1 and GRN communities (Tukey-Kramer *a posteriori* test at α=0.05) Microphytobenthic composition (zeax/fuco) and functional diversity varied (marginally) between sediment communities ($F_{2,10}$=3.5, p=0.07 and $F_{2,10}$=3.8, p=0.06 respectively), with lowest functional diversity and zeax/fuco ratios in LTH patches and higher diversity and relatively more cyanobacteria in GRN and CHL1 patches (Figure 3.3).

Table 3.2. Photosynthetic pigments detected in natural and restored wetland sediments during this study.

Pigment	Absorption maxima on gradient A (nm)	Principal taxonomic affiliation
chlorophyll a	431.0, 619.1, 665.1	all oxygenic phototrophs
chlorophyll b	461.3, 647.2	green algae/vascular plants
chlorophyll $c_{1,2,3}$	443.6, 580.8, 631.8	diatoms
bacteriochlorophyll a	363.1, 772.6	anoxygenic photobacteria
fucoxanthin	451.2	diatoms
diatoxanthin	453.7, 484.1	diatoms
diadinoxanthin	448.7, 479.0	diatoms
myxoxanthophyll	476.5, 509.5	cyanobacteria
zeaxanthin	453.7, 481.6	cyanobacteria
? canthaxanthin	484.1	cyanobacteria
echinenone	463.8	cyanobacteria
lutein	448.7, 474.0	green algae/vascular plants
carotenes	453.7	various algae, cyanobacteria
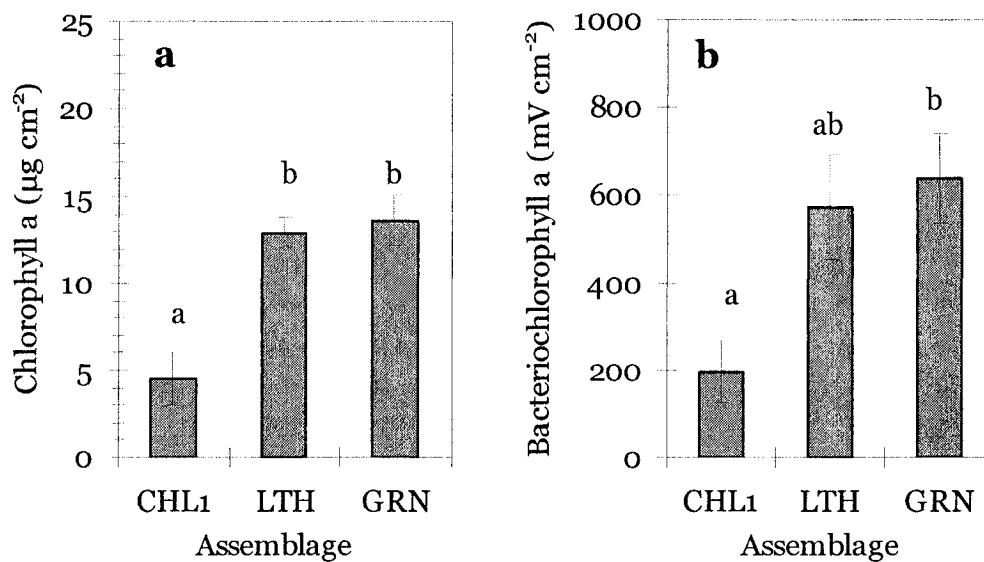
Figure 3.2. Mean (±1 S.E.) sediment concentrations of (a) chlorophyll a and (b) bacteriochlorophyll a in visually distinct benthic assemblages collected in September 2000 from restored wetland habitat. "CHL1"=macroalgal dominated sediments; "LTH"=leathery, yellowish sediments; "GRN"=greenish sediments. Similar letters designate non-significant groups of means (p>0.05). Bacteriochlorophyll a "concentrations" are in detector units.
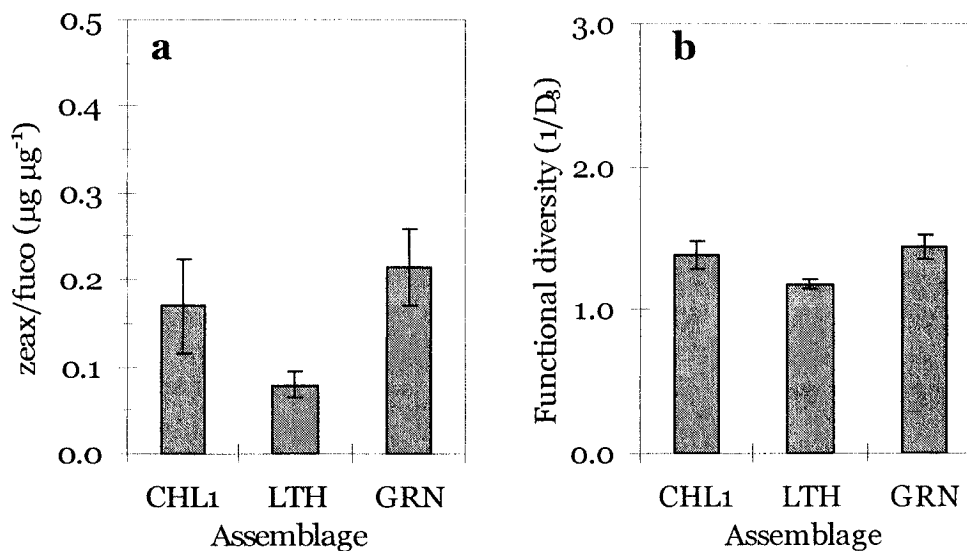
Figure 3.3. Variation in (a) cyanobacteria to diatom abundance (zeax/fuco) and (b) functional group diversity between visually distinct microphytobenthic assemblages collected in September 2000. All data are in means ±1 S.E.

In July 2001 (restored age=1.4 yr) restored wetland continued to host a variety of sediment communities; six distinct kinds of assemblages were targeted in field collections. Sediment pigment analyses revealed significant differences in chlorophyll a concentrations ($F_{5,27}$=12.8, p<0.0001; Figure 3.4), zeax/fuco ratios ($F_{5,27}$=6.5, p=0.0005; Figure 3.5), and functional group diversity ($F_{5,27}$=2.7, p=0.044; Figure 2.6) between these communities. Phototroph biomass (chlorophyll a) was highest in YEL and GRN assemblages, intermediate in BRN communities and lowest in PNK, CHL1 and CHL2 sediments. YEL and BRN assemblages possessed the highest abundance of cyanobacteria relative to diatoms. Cyanobacteria were relatively less prevalent in GRN, PNK, CHL1 and CHL2 communities. Although functional diversity was significantly different across all assemblages, *a posteriori* comparisons revealed no distinct pair-wise differences (all p>0.05).
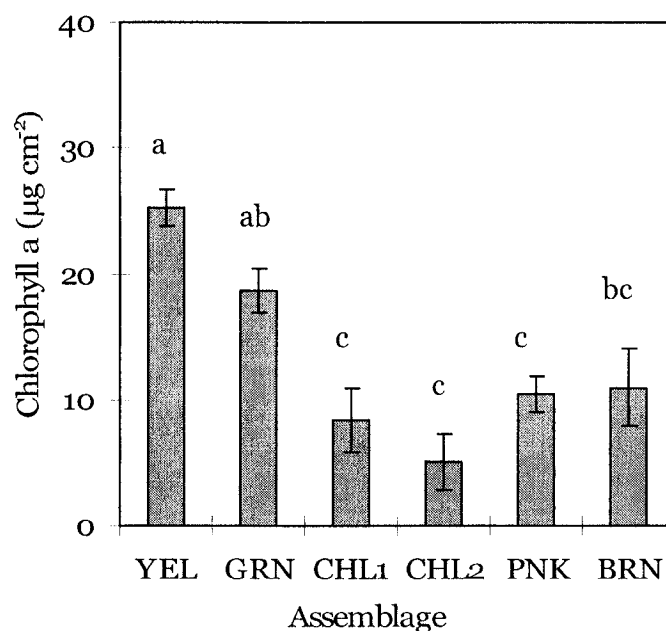
Figure 3.4. Differences in mean (±1 S.E.) sediment chlorophyll a associated with different microphytobenthic assemblages in the restored wetland in July 2001.
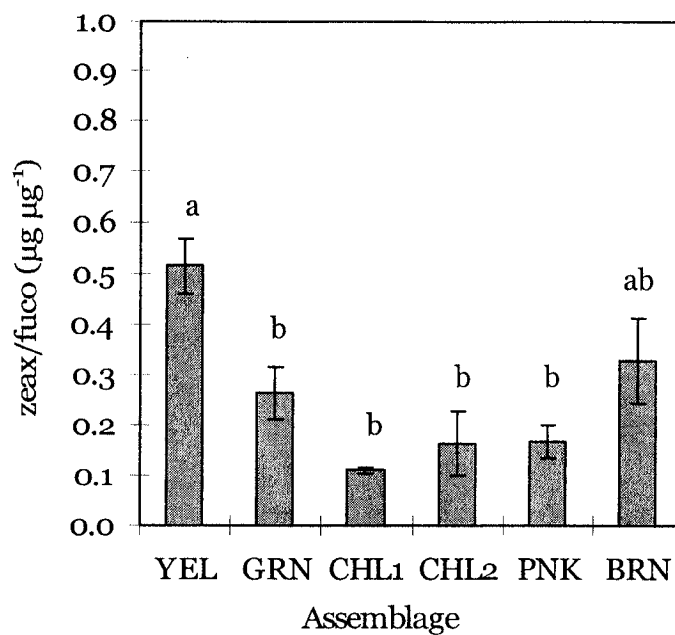
Figure 3.5. Variation in mean (±1 S.E.) cyanobacteria to diatom pigment ratios (zeax/fuco) in microproducer communities from restored wetlands sampled during July 2001.
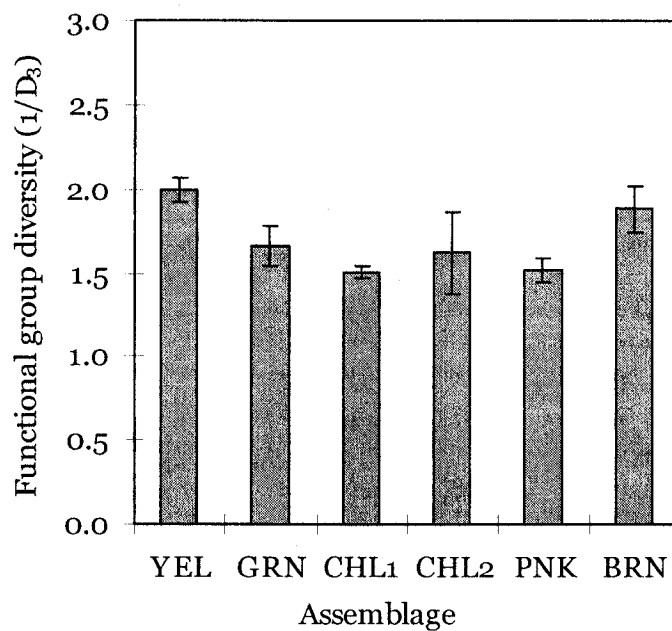
Figure 3.6. Variability in mean (±1 S.E.) functional group diversity of communities in restored wetland communities sampled in July 2001. There were no significant groups of means (all $p > 0.05$).

Long-term changes in biomass. Sediment chlorophyll a concentrations in restored wetland of both types of habitats studied rapidly matched or exceeded concentrations present in natural sediments. Two months following the opening of the restored site to tidal influence (April 2000), there was no difference in sediment chlorophyll a concentrations between restored and natural mudflats ($F_{1,10}<0.1$, $p>0.9$), but natural *Spartina* salt marsh had chlorophyll a levels about four times higher than restored *Spartina* habitat ($F_{1,10}=20.0$, $p=0.0012$; Figure 3.8). One year later (April 2001, age=1.2 yr), restored and natural mudflats again supported similar chlorophyll a ($p=0.20$), and the natural-restored difference persisted in *Spartina* salt marsh ($p=0.0006$). However, at this time, restored *Spartina* sediment chlorophyll a exceeded natural sediment concentrations by almost a factor of three. At ~1.6 yr of marsh development (September 2001), mudflat chlorophyll a was similar ($F_{1,13}=0.3$, $p>0.5$), but *Spartina* chlorophyll a differed between restored (13.1 µg cm^{-2}) and natural (4.4 µg cm^{-2}) wetlands ($F_{1,11}=13.3$, $p=0.0007$). By April 2002, sediment chlorophyll a concentrations were equivalent between restored and natural *Spartina* marsh ($F_{1,22}=3.6$, $p=0.07$) and restored and natural unvegetated mudflat ($F_{1,22}=0.1$, $p>0.7$). Finally, in spring 2003 (restored age=3.2 yr) chlorophyll a concentrations in natural and restored *Spartina* marsh were nearly identical ($F_{1,14}<0.1$, $p>0.9$).

a

b
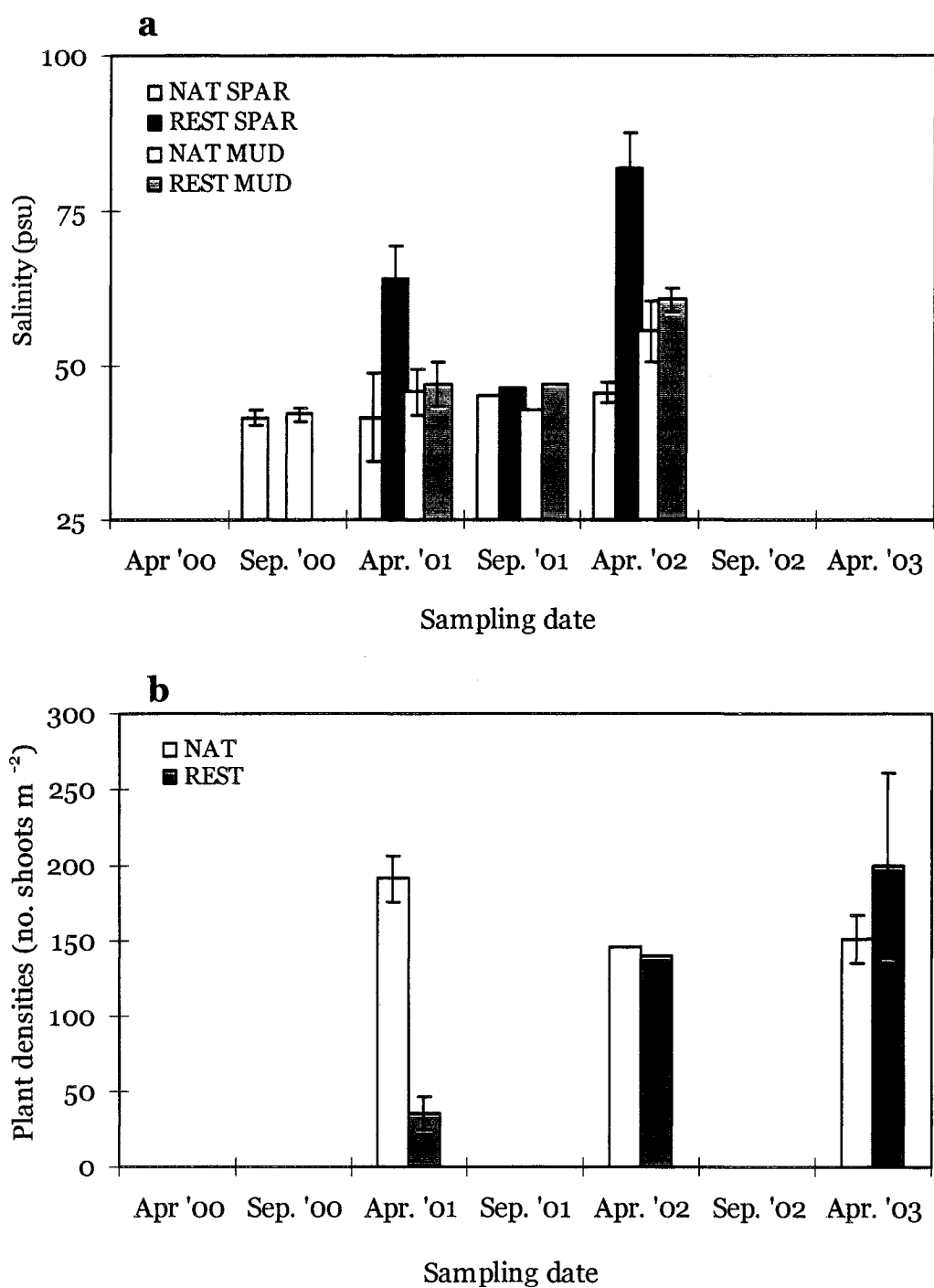
Figure 3.7. Changes in (a) mean (±1 S.E.) sediment pore water salinities in *Spartina* marsh and mudflat from 2000-2003, and (b) mean (±1 S.E.) *Spartina foliosa* densities (within *Spartina* salt marsh).
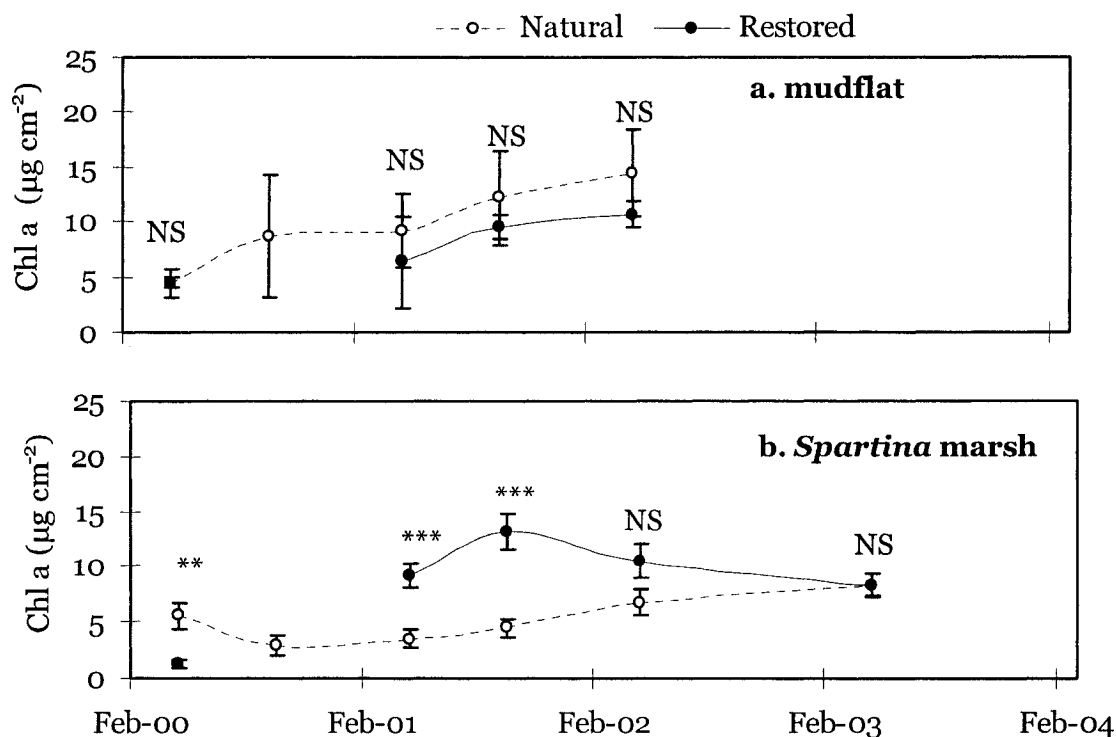
Figure 3.8. Temporal changes in sediment chlorophyll a concentrations in restored and natural (a) mudflat and (b) *Spartina foliosa* salt marsh. Restored-natural differences are indicated by: NS, not significant, * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001. The restored site was connected hydrologically to natural wetland in February 2000.

Succession of functional composition and diversity. Recovery of composition within the restored wetland was not as rapid as biomass evolution, but was largely similar to natural sediment communities by 2.2 yr of wetland development. All communities sampled early in the study (April 2000, restored wetland age=0.2 yr) were characterized by low zeax/fuco ratios and low functional diversities (Figures 3.9, 3.10) relative to later sampling periods regardless of restoration status. Nevertheless, there was a significantly higher ratio of zeaxanthin to fucoxanthin ($F_{1,10}$=16.7, p=0.002) and significantly greater functional diversity ($F_{1,10}$=12.7, p=0.005) in sediments from the natural mudflat than the restored

mudflat. There was no difference between natural and restored *Spartina* salt marsh with respect to zeax/fuco ratios or functional diversity ($F_{1,10}=1.3$, p=0.28 and $F_{1,10}=3.45$, p=0.09 respectively). Multivariate community analyses based on the relative concentrations of fucoxanthin, lutein, and zeaxanthin generally confirmed the univariate patterns: restored sediments were distinctly different from natural sediments in mudflat habitat (p≤0.002, ANOSIM), but there was only slight evidence of an early difference in *Spartina foliosa* marsh (p=0.04, ANOSIM; Figure 3.11a, Table 3.3).

By April 2001 (restored wetland age=1.2 yr), lower zeaxanthin/fucoxanthin (p=0.0005) and lower functional diversity (p=0.0004) in microproducer assemblages persisted within restored mudflat. A highly significant difference between restored and natural mudflat habitat was also seen in the multivariate comparison of functional group composition (p<0.0001, ANOSIM; Figure 3.11b; Table 3.3). No difference in zeaxanthin/fucoxanthin (p=0.15), functional group diversity (p>0.6), or the multivariate analysis of composition (p>0.3, ANOSIM) was evident during April 2001 for *Spartina* salt marsh habitat (Figure 3.11b). Differences in community composition and diversity between restored and natural mudflat persisted into September of 2001 (restored age=1.7 yr). Again, restored mudflat communities hosted less cyanobacteria relative to diatoms ($F_{1,21}=7.6$, p=0.012) and had lower functional group diversity ($F_{1,21}=11.3$, p=0.003) than natural sediments. Multivariate measures of composition confirmed mudflat differences (p=0.005, ANOSIM). However, like April 2001, there was no difference in zeax/fuco ($F_{1,16}=0.8$, p>0.3), multivariate measures of composition (p>0.3, ANOSIM), or functional diversity ($F_{1,16}<0.1$, p>0.9) between restored and natural *Spartina* microproducer communities.

By April 2002 (restored wetland age=2.2 yr), zeax/fuco ratios, functional

diversity and multivariate measures of composition were similar between restored

and natural mudflat (zeax/fuco: $F_{1,21}<0.1$, $p>0.9$, and $p>0.2$, ANOSIM) and between

restored and natural *Spartina* marsh (zeax/fuco: $F_{1,20}=3.3$, $p=0.08$, and $p>0.3$,

ANOSIM; Figure 3.11c). Additionally, functional group diversity was also essentially

equivalent between sites ($F_{1,21}<0.1$, $p>0.9$ for mudflat and, $F_{1,20}=3.6$, $p=0.07$ for

Spartina habitat). During the final sampling period (April 2003, when restored

marsh was 3.2 yr), *Spartina* sediment zeax/fuco ratios and functional diversities were

substantially depressed relative to previous sampling dates across habitats.

Nevertheless, minor but significantly elevated zeax/fuco ratios ($H_1=6.4$, $p=0.01$,
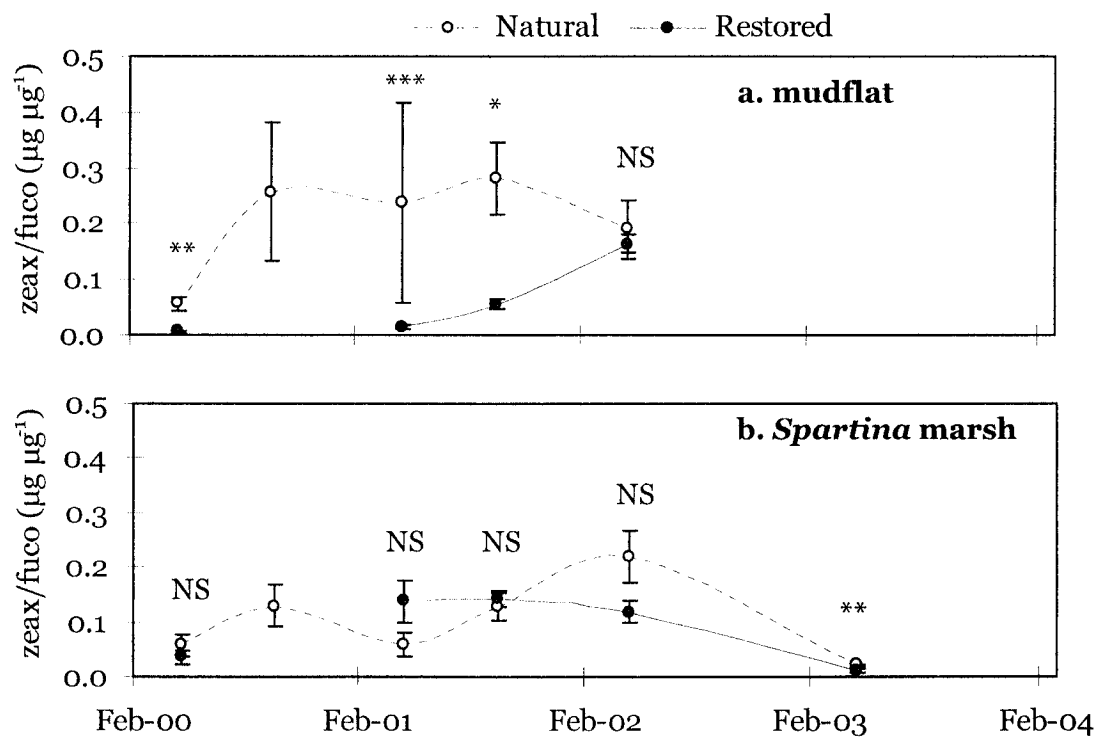
Figure 3.9. Temporal changes in the mean ($\pm$ 1S.E.) abundance of cyanobacteria relative to diatoms (zeax/fuco) in natural and restored sediments in (a) mudflat and (b) *S. foliosa* salt marsh. Restored-natural differences are indicated by: NS, not significant, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, **** $p\leq0.0001$.

Kruskall-Wallis test) and functional diversities ($H_1=7.5$, p=0.006, Kruskall-Wallis

test) were observed in natural *Spartina* marsh relative to the restored site.

Multivariate analyses of community composition also suggested a significant restored

versus natural wetland difference (p=0.0003, ANOSIM; Figure 3.11d; Table 3.3).
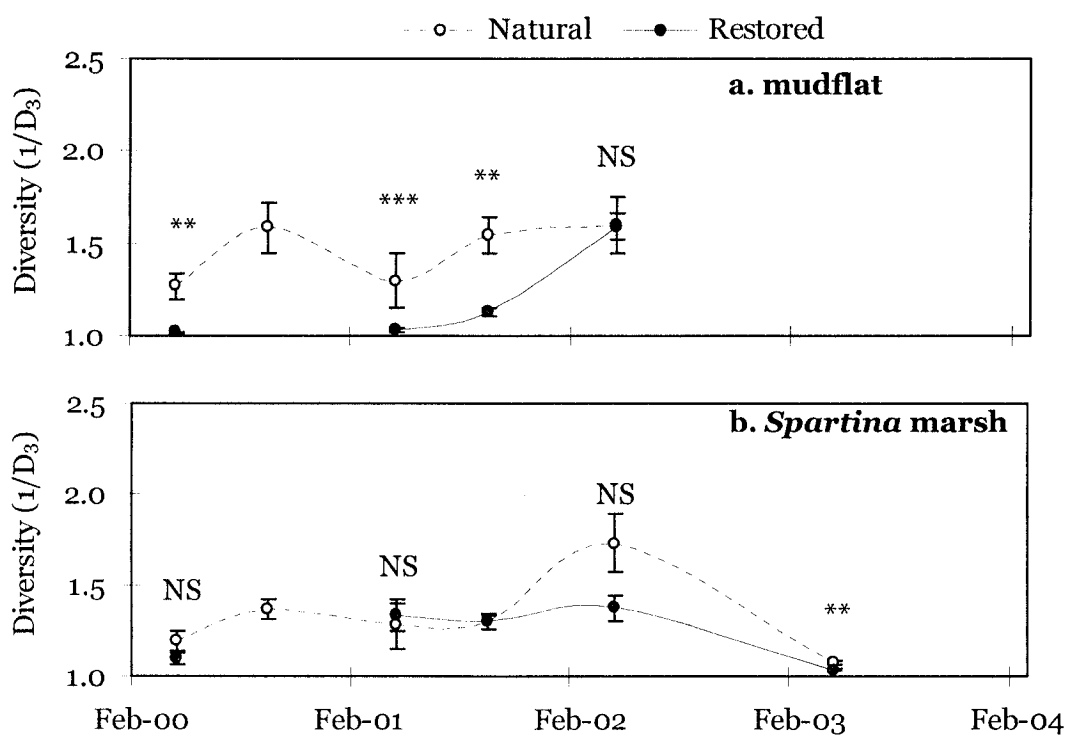
Figure 3.10. Succession of microphytobenthic functional group diversity in (a) mudflat and (b) *Spartina* habitats. Mean (±1 S.E.) diversities based on Simpson's index ($1/D_3$) incorporating lutein, zeaxanthin, and fucoxanthin. Natural versus restored differences are indicated by: NS, not significant, * $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$, **** $p \le 0.0001$.

a. April 2000 (REST age=0.2 yr)

b. April 2001 (REST age=1.2 yr)

□ NAT Spartina (n=6)
■ REST Spartina (n=6)
△ NAT mudflat (n=6)
▲ REST mudflat (n=6)

□ n=11
■ n=11
△ n=9
▲ n=16

c. April 2002 (REST age=2.2 yr)

d. April 2003 (REST age=3.2 yr)

□ n=12
■ n=10
△ n=12
▲ n=11

□ n=8
■ n=8
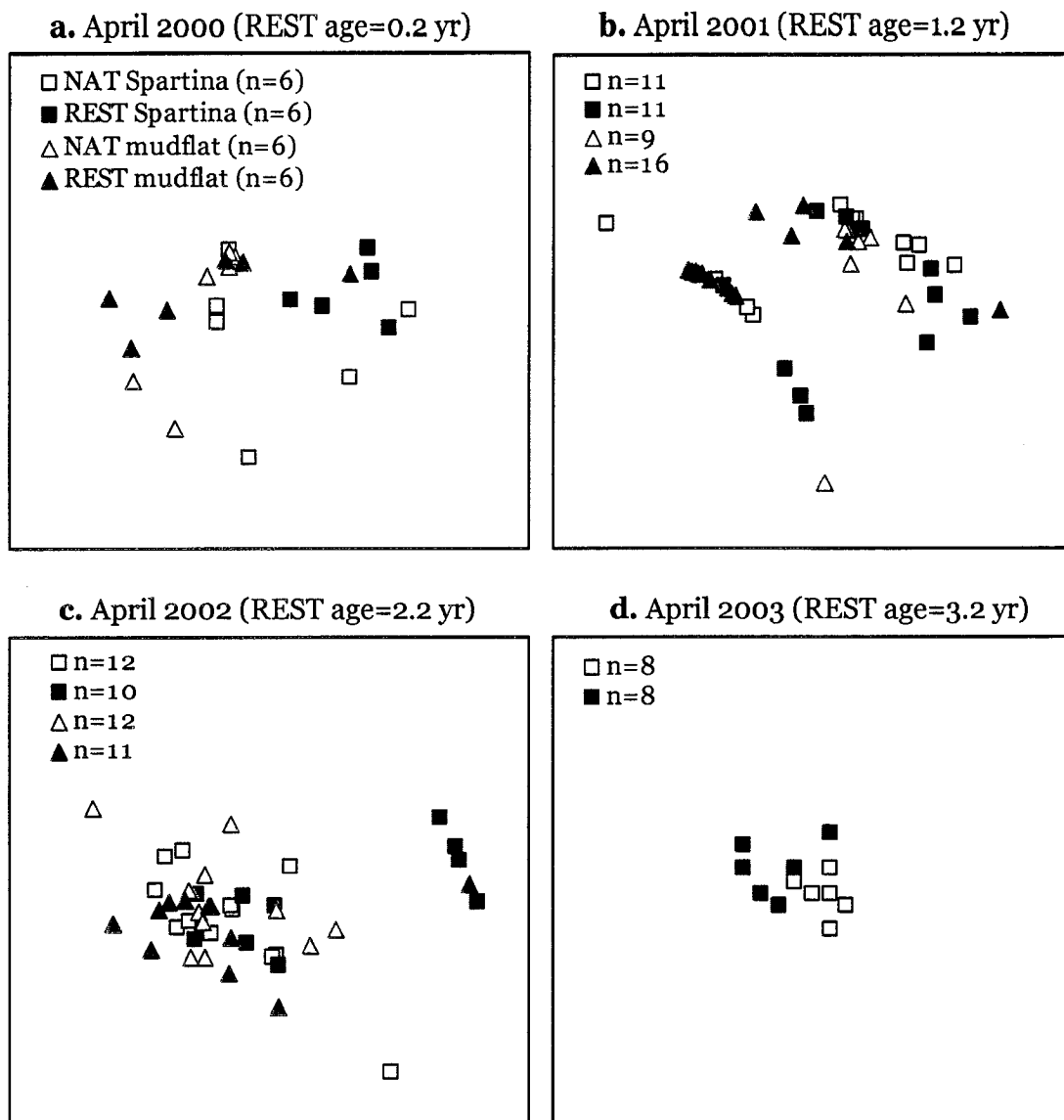
Figure 3.11. Differences in sediment microproducer community composition between restored (REST) and natural (NAT) wetlands in (a) April 2000, (b) April 2001, (c) April 2002, and (d) April 2003. Individual communities were separated in two dimensional space by nMDS analyses based on relative pigment concentrations (fuco/B_3, lute/B_3, and zeax/B_3). Distances between communities are proportional to differences in composition. April 2000, stress=0.03; April 2001, stress=0.05; April 2002, stress=0.01; and April 2003, stress=0.01. Some individual communities may not be visible due to overlap; one outlying community from April 2003 is not shown.

Table 3.3. Comparison of microphytobenthic community composition between natural (NAT) and restored (REST) mudflat and *Spartina* marsh by multivariate pigment abundances. Community composition was represented by the relative concentrations (assemblage dominance) of fucoxanthin (fuco), lutein (lute), and zeaxanthin (zeax). lute, fuco, and zeax were each scaled to L_3, where B_3=fuco+lute+zeax. Compositional homogeneity within treatments (SIM) and compositional heterogeneity between treatments (DISSIM) was determined by SIMPER; significance levels (p) of restored versus natural dissimilarity were tested with ANOSIM. Samples sizes (n_{REST}, n_{NAT}) follow significance level. NA= not measured.

Age REST (yr)	MUDFLAT				*Spartina* MARSH			
	within REST SIM	within NAT SIM	REST vs NAT DISSIM	ANOSIM p (n_{REST}, n_{NAT})	within REST SIM	within NAT SIM	REST vs NAT DISSIM	ANOSIM p (n_{REST}, n_{NAT})
Ap. 2000 0.17	91.4%	94.9%	16.4%	**0.002** (6,6)	95.6%	92.3%	8.0%	**0.039** (6,6)
Ap. 2001 1.17	92.2%	88.7%	15.5%	**<0.0001** (16,6)	85.4%	85.1%	14.9%	0.32 (12,11)
Se. 2001 1.6	93.5%	87.0%	15.3%	**0.005** (9,14)	92.0%	92.2%	8.4%	0.30 (8,10)
Ap. 2002 2.2	95.7%	86.9%	9.2%	0.27 (11,12)	91.9%	87.6%	10.5%	0.31 (10,12)
Ap. 2003 3.2	NA	NA	NA	NA	95.2%	98.1%	5.7%	**<0.001** (8,8)

Microphytobenthic community relationships with environmental variables. Pigment measures of microphytobenthic community structure were compared with several environmental variables measured throughout the study. Most measures showed no correlation with community structure. Pooled data from April 2001 to April 2003 showed that sediment chlorophyll a was not correlated with restored or natural sediment pore water salinities in either *Spartina foliosa* marsh or mudflats (all $p>0.05$; Figure 3.12a,b). Zeaxanthin/fucoxanthin and functional diversity, however, were both positively associated with salinity in sediments from the restored mudflat ($r=0.46$, $p=0.005$, $n=36$ and $\rho=0.53$, $p=0.008$, $n=36$ respectively; Figure 3.12c, e), but no relationships existed between these variables and salinity within natural mudflats (both $p>0.2$) or for any of the *Spartina foliosa* habitat (all $p>0.1$; Figure 3.12d, f).

Within *Spartina* marshes, cordgrass shoot densities were compared with microphytobenthic community composition (Figure 3.12). There was no relationship between shoot densities and chlorophyll a in either natural or restored salt marsh (both $p>0.6$). In addition, shoot densities were not correlated with zeaxanthin/fucoxanthin or functional group diversity in either kind of wetland (all $p>0.1$). Like *Spartina* densities, sediment organic matter was not significantly correlated with sediment chlorophyll a or measures of composition and diversity within *Spartina* marsh (all $p>0.1$; Figure 3.13); no mudflat investigation was made.
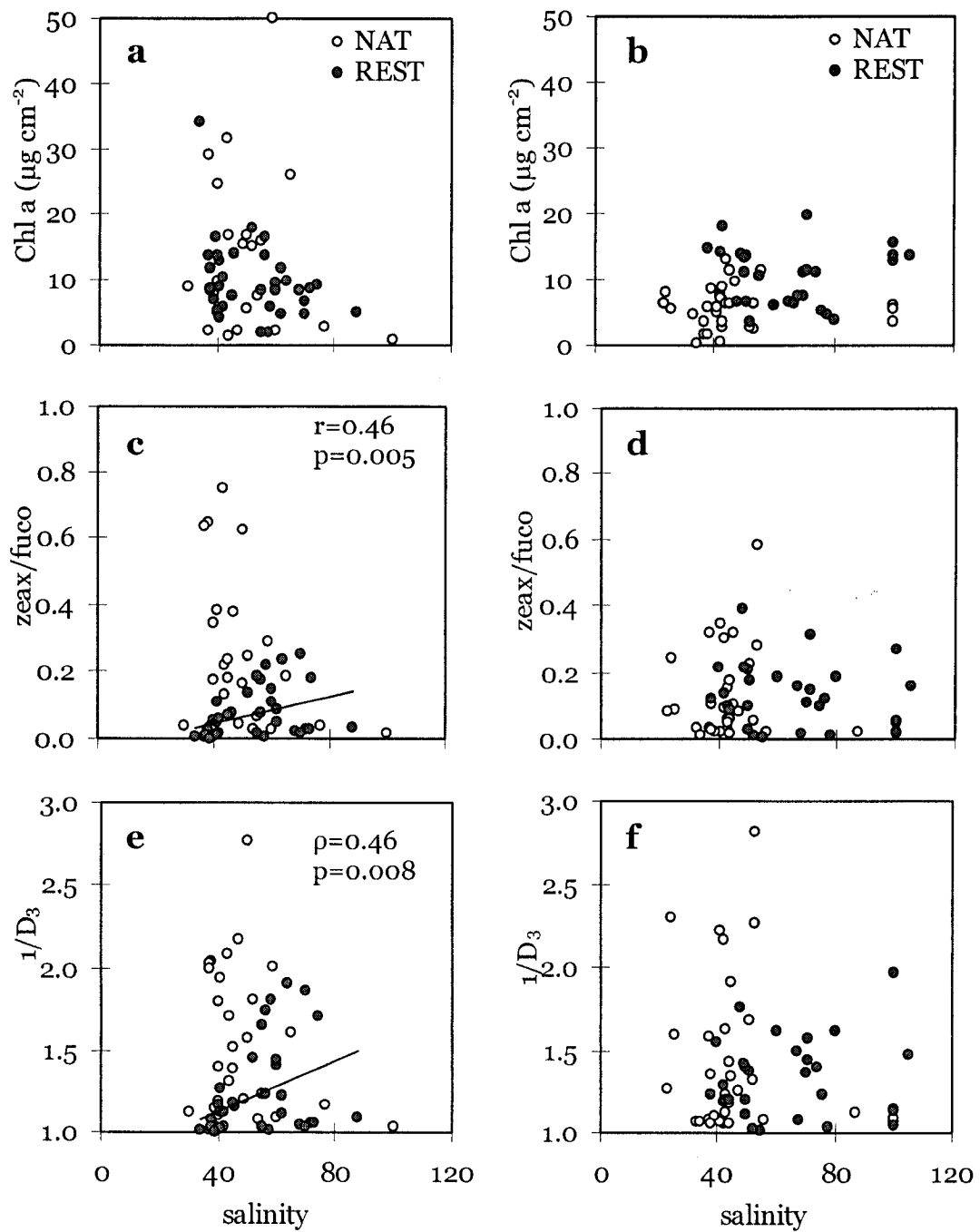
Figure 3.12. Salinity relationships with (a, b) chlorophyll a concentrations, (c, d) zeax/fuco and (e, f) functional group diversity. a, c, and e are mudflat communities; b, d, and f are *Spartina foliosa* assemblages. Significant relationships are shown in c, e.
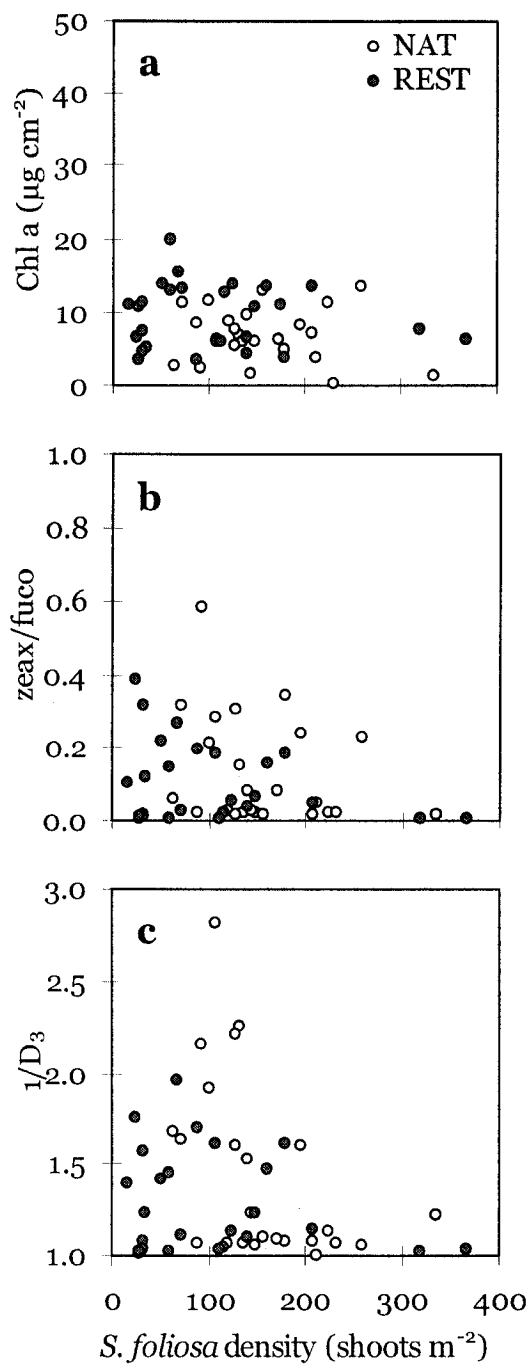
Figure 3.13. *Spartina foliosa* density relationships with (a) chlorophyll a concentrations, (b) zeax/fuco and (c) functional group diversity (all p>0.05).
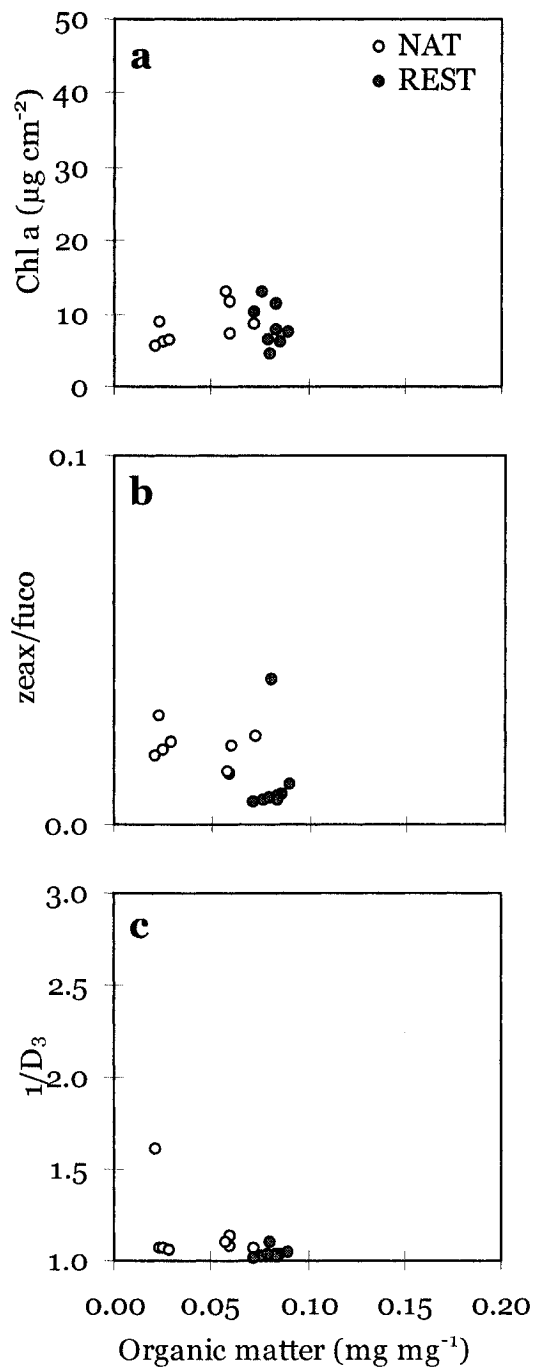
Figure 3.14. Sediment organic matter relationships with (a) chlorophyll a, (b) zeax/fuco and (c) microphototroph functional diversity (all p>0.05).

Discussion

Biomass recovery. The Friendship Marsh restoration site at Tijuana Estuary quickly acquired robust microphytobenthic populations. Despite initially low (at 2 mo) community biomass within restored *Spartina* marsh, restored chlorophyll a concentrations in both kinds of habitats studied rapidly mirrored or exceeded concentrations in natural habitat. Pigment analyses suggested that colonization within <2 mo of succession had occurred on restored sediments by diatoms and cyanobacteria in both *Spartina* salt marsh and mudflat habitats. Lower ratios of zeaxanthin to fucoxanthin suggested that diatoms were particularly common members of the early flora at the restored site and so probably accounted for the majority of chlorophyll a initially present in sediments.

Other studies that have examined microalgal colonization into new wetland habitats have also found rapid growth of microproducer populations (Underwood 1997). Zheng et al. (2004), for example, found that sediment chlorophyll a was similar between restored salt marshes of variable age and their paired reference systems. In contrast, epiphytic biomass (on vascular plants) recovered only after about a decade at their sites. Various lines of evidence from other studies confirm the high intrinsic capability of wetland microphytobenthic proliferation: Underwood (1994) noted rapid recovery of diatom biomass within days of herbicide application to sediment, and Williams (1964) documented very high population growth rates in benthic diatoms.

In any wetland restoration site initially free of resident consumer populations, rapid accumulation of microphytobenthic biomass might be stimulated by low densities of invertebrate consumers. For example, within the Friendship Marsh,

Moseman et al. (2004) found that infaunal densities approached those of natural habitat after about 1 yr. It is possible that grazing pressures were low during the first year of Friendship Marsh succession. However, it should also be pointed out that some studies have found little or only moderate effects of invertebrate consumption on standing stocks of microproducers (Page et al. 1992, Posey et al. 1995). Thus, it is also possible that early control (within the first 1-2 yrs of succession) of microproducer standing stocks was largely regulated by bottom-up processes such as nutrient availability (e.g., Armitage and Fong 2004).

Temporal evolution of composition and diversity. Restored marsh sediments rapidly acquired (by September 2000, restored site age=0.6 yr) visually distinct microproducer communities on surface sediments characterized by relatively unique pigment signatures. Pigments suggested that green algae (or plant detritus), cyanobacteria, and anoxygenic phototrophs were all present within the first 7 mo of restored marsh development. These communities included surface deposits of green algae (characterized by lower overall chlorophyll a), yellowish leathery sediments of higher biomass but relatively few cyanobacteria, and greenish sediments of both high biomass and a relatively high proportion of cyanobacteria (Figures 3.2, 3.3). The following summer (July 2001, restored age = 1.4 yr), a variety of sediment communities were again present within the restored site. Emergent (drier) sediments in upper intertidal mudflat included pinkish communities and brownish sediments with no obvious visual indication of abundant microalgae that nevertheless had moderate concentrations of chlorophyll a (Figure 3.4).

Microphytobenthic patches of distinct character may develop in wetland sediments in response to small-scale environmental features. In particular, the presence of shallow, high-salinity pools appeared to be associated with patches high

in cyanobacterial populations. Pink mats were found almost exclusively at high intertidal elevations. Pooled salinity and zeax/fuco data suggested that community composition shifted to relatively greater cyanobacterial dominance in more saline sediments with restored mudflats, but this association was curiously absent from restored *Spartina* marsh and from natural wetland of either habitat type.

Despite the general rapidity of microproducer recovery found in this study, there were habitat-specific differences in the rate at which restored wetland communities resembled natural wetland assemblages. According to compositional (zeax/fuco, multivariate analyses of dominance) and diversity ($1/D_3$) measures, restored mudflat communities took at least 1.5 yr before closely resembling natural mudflat. During this early period, natural mudflat communities were consistently more enriched in cyanobacteria and had higher functional group diversities. In contrast, recovery of composition and functional diversity appeared to be much more rapid (partially recovered by 0.2 yr) within restored *Spartina* marsh. At this point during succession (April 2000) there was only a tendency towards lower diversity ($p=0.09$) and evidence for only minor compositional heterogeneity ($p=0.04$, multivariate ANOSIM) between restored and natural SPAR (there was no difference in zeax/fuco: $p>0.2$). Natural and restored *Spartina* marsh showed little evidence for meaningful differences later on during wetland development (except for significantly higher bacteriochlorophyll a concentrations in the restored wetland during April 2002; see Figure 2.3 in Chapter II). At 3.2 yr of development (April 2003) there was significantly lower diversity and significantly less cyanobacteria relative to diatoms in restored *Spartina* marsh than in natural marsh, but this late disparity may have been related to a substantial sedimentation event that following heavy rains that spring. Indeed, zeax/fuco and functional diversity in spring 2003 were depressed across both

sites relative to spring 2002 and the restored-natural differences, though significant, were small in magnitude.

Like many other wetland investigations (Chapter II, Zheng et al. 2004), diatoms were frequently the dominant functional group of the sediment microfloras analyzed throughout this study. Zeaxanthin concentrations suggested that occasionally communities contained a substantial proportion of cyanobacteria (esp. GRN communities of September 2000 and GRN and YEL communities of July 2001 in the restored marsh), but in general, zeaxanthin was much less abundant than the diatom carotenoid fucoxanthin. Green algae were relatively rare in this study – partly because the sampling scheme of our natural-restored comparisons avoided macroscopic Chlorophyta patches, but probably also because these organisms tend to occur in localized patches that probably do not cover a large fraction of the total sediment surface.

Evaluation of restored wetland function. Observation of rapid recovery and the presence of distinct spatial heterogeneity in microphytobenthic communities in the restored site are positive indications of recovery of this wetland in Tijuana Estuary. These results suggest that (a) levels of microalgal biomass comparable to natural wetland habitat were present early in the restored site for support of invertebrate and vertebrate consumers that might use restored habitat and (b) all major functional groups were present in the restored wetland flora during both early and later succession. In particular, the presence of abundant cyanobacteria in at least some areas of the restored wetland suggests that the capacity for ecosystem processes supported by these organisms (nitrogen fixation, anoxygenic photosynthesis) would also be present.

However, these data do not imply that rates of ecosystem processes in restored habitat were equal to those of natural habitat. For example, levels of microphytobenthic consumption by invertebrates at the restored site might be much lower than in natural habitat, despite the generally similar levels of biomass. Other studies have noted the often relatively weak effects of grazers on ambient biomass (Armitage and Fong 2004), such that biomass alone reveals little about herbivory. Other processes such as nutrient fluxes, organic matter accumulation, frequency of disturbance, and photosynthetic rates may also differ between restored and natural wetland sites. Langis et al. (1991), for example, found rates of nitrogen fixation were several times lower in a San Diego Bay restoration site compared with a nearby natural wetland. Thus, although static measures of community structure are important tools for gauging ecosystem recovery (and may provide some clues about ecosystem functions), they are insufficient measures of functional processes in restoration work. Despite this limitation, it is clear that a functional group perspective on microproducer community composition will provide more information than could be gained from routine measures of sediment chlorophyll a in tracking wetland succession and recovery.

These results at Tijuana Estuary also suggest that different wetland habitats may recover at different rates. Surprisingly, I found that recovery of microproducer composition and diversity was more rapid in *Spartina* salt marsh than in mudflat, even though initial *Spartina* planting did not mimic natural habitat and plant densities took some time to match the natural system. Restored mudflat took between 1.5 and 2.2 years before attaining the levels of functional diversities and relative cyanobacterial abundance that were observed in natural mudflat. These habitat-level differences in succession may be accounted for by several factors. First,

natural mudflats in the southern arm of Tijuana Estuary are closer to intertidal channels, more extensive regions of vegetated marsh, and are generally smaller in size than mudflat from the restored site. These mudflats may receive substantial inputs of cyanobacteria from vegetated salt marsh assemblages. Second, haphazard sampling may have resulted in the selection of communities closer to vegetated sediments in the natural marsh than in the restored marsh where mudflat communities were more influenced by the higher cyanobacterial content of vegetated salt marsh. Finally, restored wetland epibenthic consumers (*Cerithidea*) may have been higher in restored mudflat than in natural habitat, preferentially consuming cyanobacteria (Armitage and Fong 2004).

Management implications. In addition to the assessment of plant, invertebrate, fish, and bird populations, future mitigation work in intertidal marine wetlands should track the recovery and temporal dynamics of benthic microalgal communities. Because they are a principle source of ecosystem-wide primary production in wetlands (Figure 1.1, Kwak and Zedler 1997), the status of microphytobenthic assemblages is likely to have cascading effects on other trophic levels colonizing or transiting a restoration site. Microproducer composition may also indicate (or provide additional evidence for) changes in the nutrient status and organic matter or oxygenation of sediments. Succession should be monitored in conjunction with at least one (preferably more) natural reference wetlands to assess how absolute population densities, relative measures of composition, and changes in the heterogeneity (e.g., population variance) of restored communities compare with undisturbed sites.

To routinely study sediment microproducer succession, I suggest that a functional group approach (via techniques such as pigment diversity or rRNA

analyses) provides several advantages. First, it provides valuable information on compositional and diversity aspects of recovery, but can be completed more rapidly than intensive microscopic inventories of microfloras (where taxonomic difficulties and time-consuming electron microscopy may prohibit comprehensive study). Since microproducer assemblages are so variable at multiple spatial scales (and highly patchy), high replication is often needed to adequately characterize communities. Second, a functional group approach provides enough taxonomic resolution to be useful for tracking the successional trajectories of particular groups that may be sensitive to minor differences in habitat characteristics (although here a species level approach might be most desirable). Finally, a functional group approach can provide at least limited information on the functional capacity of restored communities. Coupled with measurement of carbon and nitrogen fixation rates, and other aspects of food web dynamics, characterization of functional group composition provides a comprehensive perspective on restoration recovery.

To rapidly promote robust microphytobenthic communities in marine restoration work, it may be important to provide ample variation in habitat structure via sediment topology and vascular plant distribution. Microphytobenthos are believed to be very widely distributed throughout intertidal wetlands (Sage and Sullivan 1978), but because some taxa appear to have affinities for certain kinds of microhabitats (e.g., Zedler 1982), habitat heterogeneity would ensure the greatest possible heterogeneity in microphytobenthic composition in early restoration. For example, provision of shallow intertidal pools (this study) and vascular plant canopies (Chapter II) may provide more ideal microhabitats for cyanobacteria than flat, well-drained mudflats. Additionally, since restored wetlands are often depauperate in nutrients and organic matter long after their initial establishment (Craft 2000), it

may be preferable to enhance the success of cyanobacteria and anoxygenic phototrophic bacteria by seeding sections of wetland habitat with detrital organic matter.

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Chapter IV.

Functional diversity and productivity in marine wetlands:

Niche separation versus redundancy in microphototrophs

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Abstract

Ecosystem-level processes such as primary production can be influenced by the biological diversity present in communities. In coastal marine wetlands, benthic algal and bacterial producers play key functional roles in community energy fluxes, including the provision of a substantial proportion of total ecosystem carbon fixation via oxygenic and anoxygenic photosynthesis. However, little is known about the redundancy of the major taxonomic groups in shallow water sediments or whether diversity could enhance function in these assemblages. Here I present the first detailed investigation of the effects of functional group diversity and dominance on short-term productivity rates (gross oxygenic photosynthesis) and diversity/dominance relationships to microproducer standing stocks. I also tested the hypothesis that diversity-biomass relationships would differ qualitatively between disturbed and undisturbed habitats.

In naturally-occurring wetland assemblages in coastal southern California, chlorophyll a-normalized gross photosynthetic rates were not significantly affected by variation in functional diversity or functional group dominance in sediments from either salt marsh creek-bank habitat or vegetated marsh sediments. Biomass was also unrelated to functional diversity in the creek-bank assemblages, but was weakly positively correlated with diversity in sediments from salt marsh. In this wetland habitat, higher microphototroph biomass was associated with greater anoxygenic phototroph dominance and reduced diatom dominance in vegetated marsh, but overall, dominance was poorly related to community photosynthetic rates. Laboratory-constructed assemblages of three major wetland taxa (diatoms, cyanobacteria, and green algae) showed substantially higher gross productivity than

replicate cultures consisting of only cyanobacteria but, because of higher rates of green algal and diatom production, I found no overall diversity effect on photosynthetic rates. Finally, evidence from several different marine wetland habitats in two southern California sites showed that diversity-biomass relationships in restored (disturbed) habitats were often statistically similar to relationships from natural (undisturbed) wetlands, suggesting that enhanced diversity is usually unrelated to biomass across most marine wetland habitats. However, when significant differences were observed, restored relationships tended to be more negative than those of natural wetland. Although diversity did not generally appear likely to affect microproducer productivity, because each major taxonomic group may favor distinct environmental conditions, fill distinct ecological niches, and perform specialized ecosystem functions (e.g., nitrogen fixation), the presence of all taxonomic groups is probably necessary to maintain functional capacity in this patchy, variable, and physiologically stressful ecosystem.

Introduction

Diversity and ecosystem function. Previous ecological debates about the relationship between the complexity and stability of biological communities (e.g., MacArthur 1955, Elton 1958) have resurfaced recently in the form of a vigorous discussion of the effect of changes in biodiversity on ecosystem-level processes. Studies conducted within the last decade have moved beyond the earlier anecdotal and modeling efforts linking complexity to stability towards manipulative experiments which test the effects of variable diversity on primary production (e.g., Tilman et al. 2001, Engelhardt and Ritchie 2002), rates of community respiration and

decomposition (Naeem et al. 1994, Wohl et al. 2004), nutrient dynamics (Hooper and Vitousek 1998, Emmerson et al. 2003), abiotic habitat characteristics (Bolam et al. 2002), community invasibility (Stachowicz et al. 1999, Levine 2000, Symstad 2000), or the trajectory of community succession (Allison 2004).

Within this quickly evolving field of community ecology, terrestrial grassland plant assemblages have been the most thoroughly studied group of organisms to date. In large-scale manipulations of species or functional group richness, for example, Tilman et al. (1996, 2001), Hector et al. (1999), and Reich et al. (2004) found that higher diversity treatments resulted in the accumulation of greater plant biomass. Diversity and productivity have also been shown to be positively associated in manipulations of plant richness in freshwater wetlands (Engelhardt and Ritchie 2002).

Since the earliest experimental manipulations by Tilman and Downing (1994) and Naeem et al. (1994), numerous theoretical arguments and models have been proposed to explain the mechanisms behind diversity effects on ecosystem processes. In particular, positive diversity-function relationships have been usually explained by either the higher probability of inclusion of a highly functioning taxon in a diverse assemblage (the "selection effect") or by the more efficient utilization of a pool of limited resources by more phenotypically diverse communities ("niche complementarity"; Tilman and Lehman 2001). However, additional mechanisms could lead to positive diversity-function relationships. For example, positive interactions (e.g., facilitation) which increase in strength or frequency as a function of diversity, may also lead to positive diversity effects on function (Cardinale et al. 2002). Additionally, more diverse communities exposed to a variable environment over time may "outperform" less diverse assemblages because different taxa (or

subsets of taxa) have superior performance under different conditions or at difference times (a phenomenon termed the "insurance hypothesis" [Yachi and Loreau 1999]) and diverse communities thus have higher levels of time-integrated function (Hooper and Vitousek 1998). Finally, the spatial analogue of the insurance hypothesis might apply in some investigations: greater habitat heterogeneity at larger spatial scales may promote positive diversity-function relationships (Cardinale et al. 2000).

In contrast to the variety of positive mechanisms proposed, higher community diversity could also lead to lower function (e.g., reduced productivity) because of a negative selection effect, whereby competitive dominants also happen to contribute less to a given function than other taxa (Tilman 2000). Additionally, if the effects of environmental variation on function outweigh the influence of diversity, any number of relationships between diversity and ecosystem function are possible (Loreau 2000). Differences in disturbance regime (Mayer and Galatowitsch 2001, Cardinale et al. 2004) or stress (Mulder et al. 2001), might alter the relationship between diversity and ecosystem processes. Fundamentally, several factors will simultaneously determine how biodiversity influences overall ecosystem function: the nature and strength of interactions between individual species and functional groups in a community, the effects of local environmental conditions on those interactions, and the spatial scale of investigation (Cardinale et al. 2000, Levine 2000, Fridley 2002).

Empirical research in marine ecosystems. In contrast to the tight focus on diversity-biomass relationships in small-scale plant assemblages within terrestrial ecology, in marine ecosystems, empirical research on how biodiversity influences function has been scattered across a variety of habitats, organism guilds, and ecosystem processes. A few have investigated diversity effects on productivity. Paine

(2002) found that in intertidal kelp assemblages, density of the highly productive genus *Alaria* was more important to intertidal productivity than species richness. In experimental work with vascular plants used during repopulation of a marine wetland restoration site, Zedler et al. (2001) and Callaway et al. (2003), found measurable diversity impacts on various ecosystem functions (including plant standing stocks), but also found that certain species were specialists at certain functions. Observational comparisons of phytoplankton production and diversity have showed alternatively negative (in coastal, nutrient rich waters), positive (in nutrient poor coastal waters), or positive but asymptotic (tropical waters) relationships between diversity and productivity (Agard et al. 1996, Vadrucci et al. 2003). Other wetland plant studies suggest no relationship between biomass and diversity of freshwater to marine vascular plants (Gough et al. 1994) or positive (but saturating) species richness along a gradient of increasing aboveground biomass (Grace and Pugesek 1997).

Other marine work has addressed diversity effects on ecosystem-level processes other than primary production. The work of Stachowicz et al. (1999) showed that more diverse assemblages of benthic invertebrates were more resistant to invasion by exotic taxa than species-poor communities, apparently because communities of higher richness were more efficient at utilizing a limiting resource - space. The studies of Bolam et al. (2002) and Emmerson et al. (2003) provide only minimal evidence for a positive effect of sediment infaunal diversity on the majority of ecosystem attributes (e.g., sediment characteristics and nitrogen and phosphorus advection) they measured. However, a microcosm-based test of benthic invertebrate diversity on similar benthic processes revealed important diversity effects on function (Waldbusser et al. 2004). Finally, a few studies have examined static relationships between the diversity of marine primary producers and the diversity of heterotrophic

organisms but no strong correlations have been observed (Irigoien et al. 2004, Parker et al. 2001). In general, the marine work conducted so far suggests some functional dependence on biodiversity but it also hints that in some communities, composition might impact a given ecosystem process more than absolute levels of species or taxon richness.

Wetland microproducers. In coastal marine wetlands, benthic algal and bacterial producers ("microphytobenthos" or "microproducers") form an important component of food webs. These organisms, which include diatoms, cyanobacteria, green algae, anoxygenic phototrophic bacteria, and other eukaryotic organisms (Sullivan and Currin 2000; see also Appendix 1), are food sources for wetland invertebrates and fishes (Page 1997, Kwak and Zedler 1997, Buffan-Dubau and Carman 2000). Additionally, microproducers contribute to the stabilization and accretion of sediments (Coles 1979, Grant and Gust 1987, Austen et al. 1999), regulation of nutrient fluxes (Tyler et al. 2003), fixation of atmospheric nitrogen (Carpenter et al. 1978), and the oxidation of sediment sulfide (Stal 2000). At one southern California salt marsh site, primary production by microphytobenthos equaled or exceeded production by the vascular plant canopy (Zedler 1980), underscoring the central role that these organisms can play in certain coastal sedimentary communities.

Sediment assemblages of algae and photosynthetic bacteria represent associations of phylogenetically unrelated organisms which share few overall ecological similarities beyond their (usually) microscopic size, their universal need for light and nutrients, and capacity for carbon fixation. For this guild of organisms, coarse taxonomic divisions (e.g., phyla and classes) can appropriately serve to delineate major functional groups (Table 1.1) and rather cleanly coincide with

different taxon-specific photopigments (Table 2.1). Metabolic diversity, for example, between sediment microproducers is profound. Some cyanobacteria and nearly all anoxygenic phototrophic bacteria are capable of nitrogen fixation (Ormerod 1992) and members of both groups can conduct anoxygenic photosynthesis via the oxidation of sulfide or other substrates (Jørgensen at al. 1986, Ormerod 1992, Stal 2000). Diatoms, the major eukaryotic microproducer group, require silicate for the construction of external frustules, exhibit vertical migratory behavior within sediments (Pinckney et al. 1994, Sullivan and Currin 2000) and are often tolerant of wide variation in sulfide and salinity (Clavero et al. 2000). Some species show chemoheterotrophic capabilities (Admiraal 1984). Euglenoids and many cyanobacteria are also capable of limited motility, and the larger green algae (*Ulva*, *Rhizoclonium*), though not self-motile, can be passively transported around wetland environments and influence sediment oxygen concentrations (Sundbäck et al. 1996).

Given the unique combination of physiological and behavioral characteristics possessed by each microphytobenthic functional group, changes in the composition and diversity present in communities could have important consequences for ecosystem functions mediated by these producers. There is also a growing understanding that the relative abundances of different microphytobenthic functional groups differ across various spatial (Pinckney and Sandulli 1990, Paterson et al. 1998, Chapter II) and temporal scales (Pinckney et al. 1995, Underwood 1997, Chapter III), suggesting that heterogeneity in community composition and diversity could ultimately lead to spatio-temporal heterogeneity in important ecosystem processes.

No comprehensive study of benthic marine microproducer diversity and productivity has been conducted to date, although previous workers have expressed the desire to understand such relationships (Underwood and Kromkamp 1999).

Sullivan and Moncreiff (1988) provided limited evidence of a negative association between diatom diversity and photosynthesis. Furthermore, Hillebrand and Sommer (1997), working with rock-inhabiting diatoms and macroalgae, found a negative relationship between community diversity and biomass ("biovolume") in a data set that combined both nutrient enriched and control communities, but nutrient availability simultaneously influenced both diversity and biomass in their study.

In this study I examined relationships between functional diversity (diversity of major taxonomic groups) and dominance and two measures of productivity in southern California microphytobenthic communities. Natural sediments and laboratory cultures were used to test the following hypotheses: (1) greater functional diversity is associated with higher gross productivity rates and higher phototrophic biomass in natural assemblages, (2) greater dominance by individual functional groups is correlated with increasing productivity in field assemblages, (3) artificially-constructed communities composed of multiple functional groups show higher photosynthetic rates (gross production) than single functional group treatments, and (4) diversity-biomass relationships are positive in restored (disturbed) wetlands but negative in natural (undisturbed) habitats. Simultaneous measurement of productivity and diversity on field-collected material allowed the determination of *in situ* relationships at ambient diversity levels (but uncontrolled functional richness) and laboratory cultures enabled a specific test of increased functional group richness on assemblage production. From these multiple approaches insight is gained into the specific role that functional composition, dominance and diversity play in production dynamics of wetlands and the potential mechanism(s) underlying the diversity-productivity relationships in these assemblages.

Materials and methods

Natural diversity-productivity patterns: field collections. Replicate sediment cores were collected from salt marsh creek-bank habitat in the Kendall-Frost Mission Bay Marsh Reserve, San Diego County, CA, U.S.A. (32°47' N, 117°13' W) from 17 to 26 June 2002. A transparent acrylic core (10 cm length, 5 cm diameter) was pushed to ~6 cm depth at random locations along a 1 m transect selectively placed throughout the site to obtain a variety of sediment communities (hereafter "live cores") for transport to the laboratory. Surface sediment in the immediate vicinity of the live core was collected for analysis of sediment grain size and organic matter (4 cores, 21.5 mm diameter by 1 cm deep) and sediment pore water salinity (1 core, ~14 mm diameter). Salinities were estimated in the field by pressing wet sediment into a 10 ml plastic syringe fitted with ~2 paper filters and measuring the filtrate on a hand-held refractometer. Light transmission to the sediment surface at each location was estimated by 3-4 replicate measures of irradiance flux (in quanta cm^{-2} s^{-1}) made approximately 1 m above the ground and just above the sediment surface with a Biospherical QSL-100 irradiance collector (Chapter II).

Live cores were generally stored in the dark (at $\leq 30\,°C$) during transport to the laboratory. All live cores used for production and oxygen profile measurements were submerged in a slow flow-through chamber (~9.5 L capacity) situated inside an incubator (22.5°C; light flux ~70 μE m^{-2} s^{-1}) and filled with 1 μm filtered coastal seawater obtained from the Scripps Institution of Oceanography pier (final salinities in the water overlying the cores =32.5 to 35 psu). Four control cores filled with floral sand were incubated in the same chamber with field collected sediment intermittently during the experiment (and then sub-sampled for pigment work) to roughly estimate

any potential sources of contamination including settlement of live cells from filtered seawater on the sediment surface and/or movement of algae between live cores.

Live cores were again collected from July to September 2004 from random locations within salt marsh habitat in the upper intertidal of the Kendall-Frost Reserve. Thirty-three replicate cores were obtained from vegetated marsh composed of various combinations of these halophyte species: *Salicornia bigelovii, S. virginica, Batis maritima, Triglochin* sp(p)., *Jaumea carnosa, Limonium californicum, Distichlis spicata, Spartina foliosa, Monanthochloe littoralis* and *Frankenia salina.* At each location, vascular plant species richness and composition inside a 0.25 m^2 quadrat were measured and, as in June 2002 work, pore water salinities (from 0-1 cm deep surface sediments) and light transmission through the plant canopy were estimated.

Microelectrode measurements. Cores were moved from incubation at 22.5°C (with filtered seawater covering the sediment surface) onto a stand for oxygen measurements within several hours (June 2002) or within ~1.5 wk (summer 2004) of collection from the field. All oxygen measurements were obtained with fast-response time (<1 s), Clark-type glass oxygen microelectrodes (10, 25, or 50 μm terminal diameter) attached to a micromanipulator stand. Electrodes were polarized throughout the measurement period (-0.80 mV) with a Unisense PA2000 Picoammeter and calibrated each day prior to use by measuring response at 100% O_2 saturation and 0% O_2 saturation (bubbling an enclosed chamber with atmospheric gas and He or N_2 gas respectively).

Production measurements in June 2002 were initiated using the "light-dark shift" technique outlined in Revsbech and Jørgensen (1986) following a 20 min acclimation period of exposure to light (e.g., see Figure 4.1). During this period, the

microelectrode tip was positioned at a randomly determined position on the sediment surface by manual adjustment of the micromanipulator stand under magnification (generally at 12 x). The sediment surface was defined as 0 μm and subsequent movement into (as + μm) or out of (as - μm) the sediment was made using Profix 2.1 software (Pyro Imagination), an AD-101 digital/analogue interface (Pico Technology Limited), and an Oriel 18011 Encoder Mike Controller motorcontroller (vertical precision =0.1 μm; Unisense Corporation). The sediment surface was illuminated with an irradiance of 72.3 ± 3 μE m^{-2} s^{-1} (provided by Techniquip Corporation Fiber Optic Illuminators), a quantity approximately 3% of natural light flux to the reserve on a sunny spring day. After the acclimation period, light flux to the sediment surface was blocked for ~20 s and then restored. Oxygen concentrations were obtained every 2-3 s prior to and after the light was extinguished. Production measures were also obtained in a similar manner at 250 μm and 500 μm depth following a brief (~5 min) acclimation period at each depth under exposure to light. Changes in the O$_2$ concentration for ~ 2-6 s periods (or up to 20 s in cases of substantial scatter) prior to and following the loss of illumination were calculated. The slope of the light period was subtracted from that of the dark to obtain productivity rate estimates.

a

b

Productivity (μmol O_2 l^{-1} s^{-1})
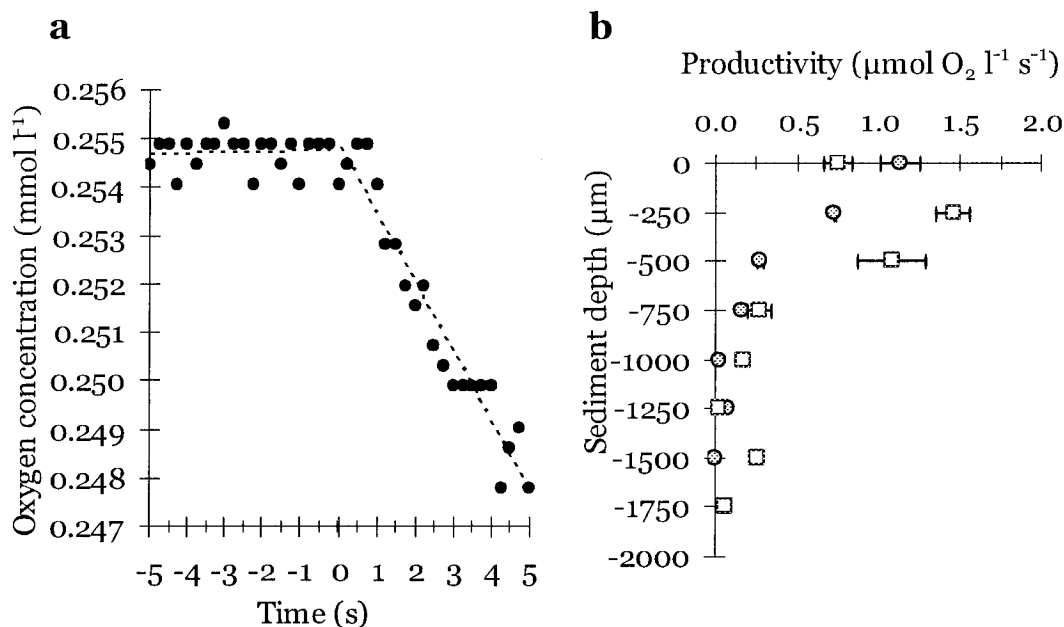
Figure 4.1. Estimation of gross oxygenic production via microelectrode measurements. (a) Temporal change in oxygen concentration before and after sediment shading. The magnitude of oxygen depletion following cessation of illumination (at t=0 s) generates a productivity estimate. (b) Depth profiles of mean ($\pm$1S.E.) gross production in two cores from mixed halophyte salt marsh (summer 2004). All photosynthetic measurements in (a) and (b) were conducted at an irradiance of ~61 μE m^{-2} s^{-1} at the sediment surface.

In June 2002, depth profiles of oxygen concentration (generally -2 mm to 3 mm depth; 100 μm intervals) in several cores were obtained under one or more of three light regimes: I_a= 72.3±3 μE m^{-2} s^{-1}; I_b= 159.5±3 μE m^{-2} s^{-1}; or I_c= darkness. Profiles under light I_a followed a 10 min acclimation period at the established irradiance; profiles under light I_b were initiated following at least a 15 min acclimation period. Temperature and salinity of the overlying water in incubating cores were made with a hand-held refractometer periodically during electrode measurements to determine core-specific oxygen saturation values (International Oceanographic Tables 1971).

In summer 2004, photosynthesis measurements were conducted on field-

collected sediment cores (n=16) as above with the following changes: (1) production was measured at 250 μm intervals from the surface into the sediment until photosynthesis rates approximated zero (often at about 750 μm depth), (2) electrodes of 10 μm, 25 μm, and 50 μm diameter were variously used, (3) oxygen concentrations were determined every 250 ms (for 10 and 25 μm electrodes) or every 1 s (for the 50 μm electrode) and (4) to more precisely determine the exact point of cessation of illumination than in the 2002 work, a light meter was coupled to Sloper 3.0 software (Pyro Imagination) via an ADC-216 digital/analogue interface (Unisense Corporation). Oxygen loss over time was calculated for a 4.5 to 8 s interval following loss of irradiance. Cores were illuminated with 61 $\mu E\ m^{-2}\ s^{-1}$ irradiance. No oxygen profiles were made with depth, but photosynthesis versus irradiance curves were constructed for two cores (Figure 4.2). Production work was attempted on additional cores but output was of sufficient quality only to approximate the deepest site of observable production and the depth of highest productivity, not depth-integrated rates.

HPLC analysis of photosynthetic pigments. Following microelectrode measurements of field-collected material, two small cores (8.5 mm diameter by ~1 cm depth) were taken from each live core (directly over or in close proximity to sites of electrode work) after carefully removing the seawater overlying the sediment. An additional core for estimation of sediment water content was taken nearby. All sediment was frozen (-83.5°C for pigment cores, -20°C for water cores) within 24 hours of collection.

Pigments were extracted in 90% acetone/10% seawater. Sediment water content was calculated by measurement of sediment mass before and after placement
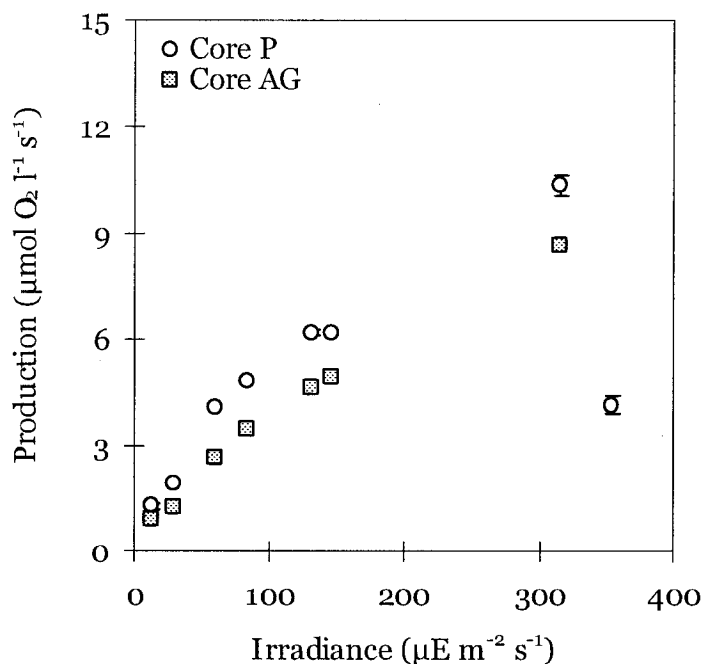
Figure 4.2. Photosynthesis versus irradiance curves for two sediment microproducer assemblages collected during summer 2004 (cores P and AG) from Mission Bay. Photosynthetic rates (all measured at 250 μm sediment depth) have not been normalized to chlorophyll a abundance here.

in a drying oven (~50-60°C). This estimate of sediment moisture for each individual core was then used to calculate an appropriate volume of HPLC-grade acetone to be added to defrosted sediment to generate a final solvent mixture= 90% acetone, 10% seawater (see Appendix 2). Samples were extracted for 24 hr in the dark on ice (~0°C). At the end of the extraction period, sediment was centrifuged and supernatant was passed through a cotton-fitted glass pipette into a clean container (Goericke 2002). Extracts were stored in the dark at ~0°C until HPLC analyses (usually within several days).

Chlorophyll a, taxon-specific carotenoids (peridinin, fucoxanthin, lutein,

zeaxanthin), and other major pigments (chlorophyll c's, diadinoxanthin, diatoxanthin, chlorophyll b, carotenes) were separated on an Adsorbosphere® C_{18} HS reverse phase column (3 μm diameter stationary phase; 10 cm length; 4.6 mm diameter) with a 40 min bi-phasic gradient modeled after Brotas and Plante-Cuny (1996) (see Gradient H in Appendix 2 for a detailed description). Pigment abundances were determined by absorbance (450 nm) and fluorescence detection (excitation=430 nm, emission=674 nm). Bacteriochlorophyll a concentrations were determined by an additional separation on an Adsorbosphere® C_{18} HS reverse-phase column (5 μm diameter stationary phase; 15 cm length; 4.6 mm diameter) with a two solvent gradient modeled after Goericke (2002) and absorption detection at 770 nm (see Gradient G in Appendix 2). For both gradients G and H, 100 μl of a 600 μl sediment extract + 250 μl water mixture was input into the system. Material was stored cold (0°C) and in the dark until analysis.

Pigments were identified by comparison of elution times in sediment material with pigments from mono-specific cultures representing diatoms (*Thalassiosira weissflogii*), cyanobacteria (*Arthrospira platensis*), green algae (*Dunaliella tertiolecta*), dinoflagellates (*Lingulodinium polyedrum*), and eustimatophytes (*Nannochloropsis oculata* for the vaucheriaxanthin-ester found in *Vaucheria*) or by comparison with semi-pure pigment (e.g., bacteriochlorophyll a from *Rhodopseudomonas sphaeroides*; Sigma-Aldrich, Inc.). For all pigment samples, detector responses were converted to pigment concentrations by calibration regressions generated with pure pigments (DHI Water and Environment, Hoersholm, Denmark). Pigment concentrations reported here are in μg pigment cm^{-2} sediment or μg pigment cm^{-3} (chlorophyll a).

Analyses of sediment abiotic features. Sediment grain size distribution

and the abundance of organic matter in sediments were determined in the laboratory according to methods outlined in Talley and Levin (2001) and Carver (1971) respectively. Briefly, sediment was separated on a 63 μm sieve into larger (sand and granules) and smaller (silt and clay) fractions and dried and weighed. Organic matter was determined by combustion of dried sediment at 550°C for several hours and subsequent calculation of mass loss (Carver 1971). For estimation of surface nitrate and ammonium concentrations (a few summer 2004 cores only), pore water in the upper 1cm of sediments was filtrated sequentially though Whatman® grade 1 paper filters and 0.45 μm Millex® LCR polypropylene syringe filters, diluted with fresh water, and then frozen (-20°C) in sealed polypropylene containers. Concentrations were determined by the UC Division of Agriculture and Natural Resources laboratory (UC Davis). Microphototrophs from halophyte salt marsh sediments were observed and photographed during summer 2004 (Figure 4.3).

Laboratory tests of richness effects on production. Algae collected from the Kendall-Frost Mission Bay Marsh Reserve were isolated from sediment and other benthic phototrophs during 2004-2005 and grown initially on f/2, f/2 minus sodium silicate, or f/4 minus sodium silicate media (www.pcc.org) prepared with artificial seawater (salt) (21-22°C, 13 hr light cycle). After the establishment of unialgal (but not axenic) cultures, stock cultures of pennate diatoms (D), the filamentous cyanobacterium (?) *Leptolyngbya* (CY), and the green alga, *Ulva* (G), were placed in full f/2 media on one side of a 30 ml glass jar or transparent tube partitioned in half by a glass particle filter (1.6 μm pores) that allowed media and bacterial circulation throughout the chamber but retention of the larger algal cells on one side.
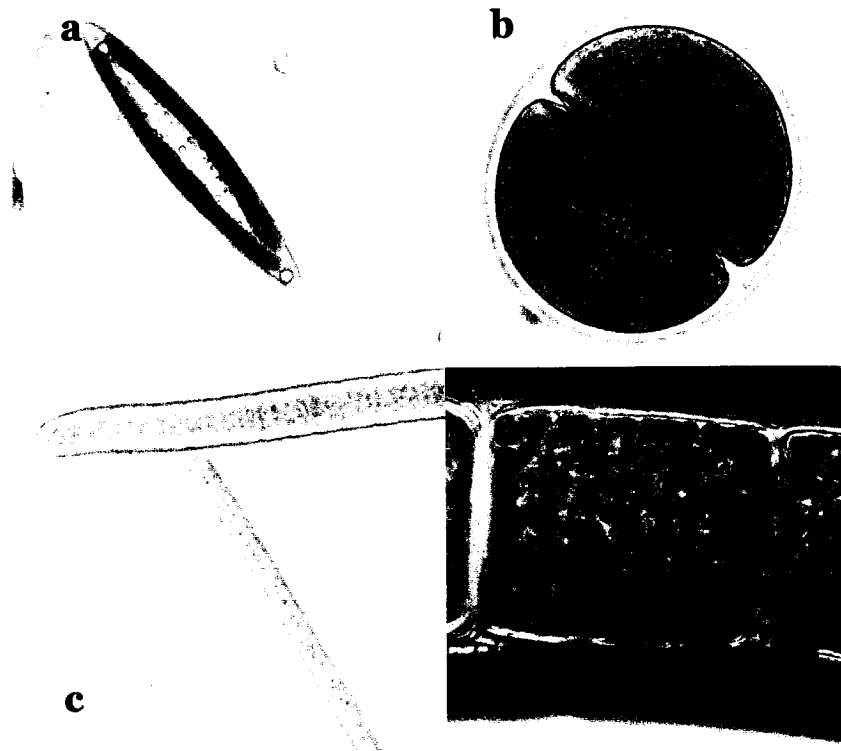
Figure 4.3. Sediment-associated benthic phototrophs collected from halophyte salt marsh in summer 2004. (a) pennate diatom *Navicula*, (b) coccoid cyanobacterium *Chroococcus*, (c) filamentous cyanobacteria *Oscillatoria* and *Microcoleus*, (d) green alga *Rhizoclonium riparium*.

In a test of functional group richness on gross photosynthetic production, replicate microcosms were constructed of one, two or three functional groups. Moderate volumes (0.5-3.0 ml) of three stock cultures (filamentous cyanobacteria, green algae, and diatoms) were added to 30 ml glass jars containing 10 ml sterile f/2 media and 10.0 g of glass particles (13 to 44 µm semi-transparent beads) to create communities of one (n=12) or three (n=8) functional groups. All single functional group treatments were replicated at least 3 times. Two replicates of cyanobacteria +diatoms (one made inadvertently by contamination) were also investigated. All experimental cultures, regardless of treatment, contained an equal final volume of f/2 media (14.5 ml), roughly equivalent initial algal biomass (1.5 or 3.0 ml) and an aliquot of media and associated bacteria from each of the three stock cultures to avoid confounding effects of differences in algal biomass, media chemistry, or bacterial composition. One to three cultures were assembled during a single day. Microcosms were haphazardly arranged inside a single illuminated incubator and were uncapped briefly for atmospheric gas exchange and haphazardly rearranged, spatially, in the incubator several times each week.

Following 8-12 days of incubation, each culture was placed on a microelectrode stand under approximately 200 µE m^{-2}s^{-1} illumination. Photosynthetic rates were obtained from the surface to 3 mm depth at 250 µm intervals with 10 or 25 µm glass oxygen electrodes. To normalize depth-integrated production rates to assemblage biomass, sub-samples of culture substrate (8.5 mm diameter by roughly 10 mm deep cores) centered over the site of production work were taken within approximately 2.5 d to determine chlorophyll a abundance. Pigments were frozen (-80°C), later extracted in 90% acetone/10% f/2 media, and separated by HPLC using gradient 'H' (see Appendix 2). Electrode work was

conducted from April to June 2005.

Estimation of diversity and dominance. In field-collected wetland sediments (June 2002 and summer 2004), concentrations of four to five taxon-specific pigments - lutein (lute), zeaxanthin (zeax), fucoxanthin (fuco), peridinin (peri), and bacteriochlorophyll a (bchl a) - were used to represent the biomass of green algae/plant detritus, cyanobacteria, diatoms, dinoflagellates, and phototrophic bacteria (largely purple sulfur and non-sulfur bacteria) respectively (Pinckney et al. 1995, Jeffrey et al. 1997, Imhoff 1999). Peridinin was only observed in trace quantities in two creek-bank cores (June 2002) but was included in diversity and biomass calculations for that date; it was not determined for summer 2004 material. Sediment concentrations of diagnostic pigments were incorporated into estimates of functional group diversity (Simpson's index: $1/D_5 = [\sum (p_p)^2]^{-1}$; Magurran 2004) and functional group dominance (p_p) where p_p is the abundance of a given taxon-specific pigment per total pigment abundance ('B_5'= zeax+lute+bchl a+fuco+peri). $1/D_4$ and B_4 represented the diversity and biomass of sediment communities (from summer 2004) lacking peridinin. Total concentrations of all functional group pigments (fucoxanthin, lutein, zeaxanthin, and bacteriochlorophyll a) per surface area of sediment ('B_4' or 'B_5') or sediment chlorophyll a concentrations (lab experiment) were employed as estimates of microproducer biomass.

Statistical analyses. Regression analyses were performed to test the influence of (a) functional group diversity and (b) functional group dominance on depth-integrated productivity rates and microphototroph standing stocks. In addition, relationships between functional diversity and (a) the depth of highest gross production and (b) greatest depth of any detectable photosynthesis were investigated to look for changes in the distribution of production within surface sediments. To

understand the relative importance of functional diversity versus abiotic features of the wetland environment to (1) gross production and (2) standing stocks of microproducers, multiple regression was employed with ambient light transmission, proportion of organic matter in sediments, and proportion of sand-sized particles (>63 μm) in sediments as independent variables. (Sediment pore water salinity was excluded as a fourth independent variable because of its strong co-variance [see Quinn and Keough 2002] with sediment organic matter [p=0.0002]). Additional single regressions revealed that neither the temperature nor salinity of seawater covering live cores at the time of production work in the lab nor the time of day at which production was measured significantly influenced productivity rates in June 2002 (all p>0.05) or summer 2004 (p>0.5). Prior to all statistical work, the normality of dependent and independent variables was tested (CoStat 6.101 package) and non-normal distributions were transformed as necessary.

In the microcosm culture experiment, integrated oxygenic photosynthetic rates (surface to 3 mm depth) were determined for each replicate and normalized to chlorophyll a abundance ($\mu g\ cm^{-3}$). Differences in (a) integrated photosynthetic rates and (b) depths of maximum photosynthetic production between one and three functional groups were tested with one factor ANOVA. Furthermore, production rates of single taxon cultures (of different functional group composition) and D+CY cultures were compared by one-factor ANOVA to identify the treatment with the highest assimilation rate ($\mu g\ C$ fixed $\mu g\ chl\ a^{-1} s^{-1}$) under the experimental conditions.

Finally, sediment pigment concentrations from previous work on the spatial distributions of diversity and composition (Chapters II, III) were utilized to test whether diversity-biomass relationships differed between disturbed (early succession in a restored wetland) and undisturbed (mature, natural) wetland habitat.

Specifically, I tested whether diversity is positively related to biomass in stressed (disturbed restored sites), but negatively or neutrally associated with biomass in natural, undisturbed habitat – patterns which would imply facilitation effects of diversity on function. Wetland microphytobenthic communities from two sites were included in the test: Tijuana Estuary (which hosted a 0 to 3.2 yr-old, 8 hectare restoration site; Chapter II) and Mission Bay (which had a small, <3 hectare, 6.3 yr-old restoration site adjacent to natural wetland; Levin and Talley 2002). Functional group diversity ($1/D_3$ or $1/D_4$) and community biomass (B_3 or B_4) relationships were determined in natural and restored assemblages separately by correlation analyses and then relationships were statistically compared between paired natural and restored wetland habitat by χ^2 tests on correlation coefficients (r). Replicate analyses were conducted for each unique site (Tijuana Estuary and Mission Bay, California), sampling period, and habitat (creek-bank/mudflats, *Spartina foliosa*-dominated salt marsh, and mixed vascular plant salt marsh) combinations. Individual relationships were considered qualitatively different at $p < 0.05$ (for r) and at $p < 0.1$ (for χ^2).

Results

Diversity-production in natural creek-bank sediments. Sediment cores collected from wetland creek-bank habitat in June 2002 were characterized by interstitial water salinities slightly elevated above normal sea water (mean: 42.5 psu) and high variability in the sandiness of sediments (8.0% to 95.0%; mean=32.1%). Light transmission to these sediments was generally high (mean=80.5%), owing to the general absence of vascular plant cover. Sediment abundances of the major taxon-specific pigments present suggested that diatoms were consistently the

dominant group (59-91% of total taxon-specific pigment concentration, B_5) with green algae, cyanobacteria, and phototrophic bacteria usually (although not always) present in sediment communities. Peridinin (from dinoflagellates) was detected in very minor concentrations (<0.01 µg cm^{-2}) in only two replicate sub-cores and was absent from all others. Photopigment abundances revealed that variation in functional group diversity (mean $1/D_5$ = 1.4; ranging from 1.0 to 2.2) largely emerged as the consequence of differences in the evenness of major taxonomic groups, not differences in functional group richness.

Functional diversity was not related to depth integrated gross productivity in creek-bank sediment microproducer communities ($p>0.2$; Figure 4.4). An 8-fold difference in photosynthetic rates (0.0008 to 0.0065 µg O_2 µg chl a^{-1} s^{-1}) was seen in assemblages of low functional diversity (when $1/D_5$ <1.9), but integrated gross production was lower and less variable at higher diversity (when $1/D_5$ >1.9). Additionally, gross production was neither associated with fucoxanthin dominance ($p=0.18$), zeaxanthin dominance ($p=0.11$), the dominance of lutein from green algae and plant detritus ($p>0.9$), nor the dominance of bacteriochlorophyll a derived from phototrophic bacteria ($p>0.5$). Furthermore, variation in functional diversity did not predict the depth (within the upper sediment layers) of highest gross photosynthesis ($p=0.15$; Figure 4.5).

Microphytobenthic standing stocks (estimated as B_5) were neither related to functional group diversity ($p>0.8$) nor to diatom ($p>0.9$), green algal ($p>0.3$), or cyanobacterial dominance ($p>0.9$; Figure 4.6) in creek-bank sediment communities. However, higher biomass was observed in assemblages that were characterized by greater dominance of phototrophic bacteria ($r=0.58$, $p=0.008$).
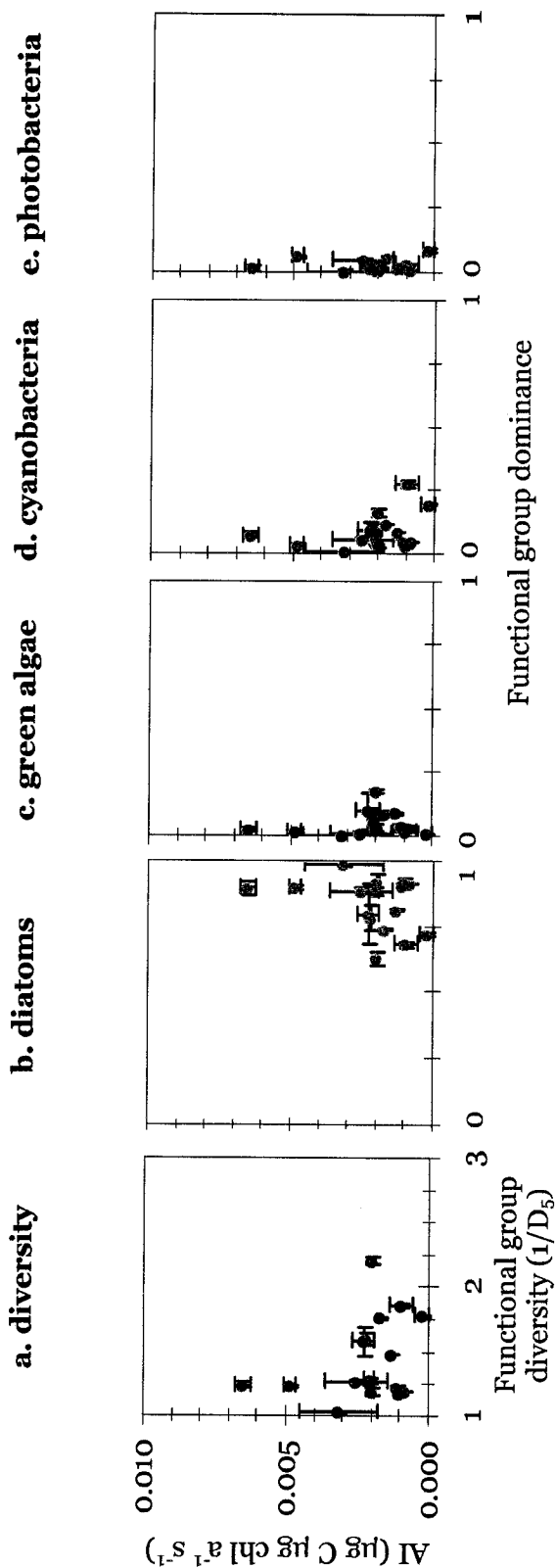
Figure 4.4. Mean (±1 S.E.) diversity (a) and mean (±1 S.E.) functional group dominance (b-e) effects on mean (±1 S.E.) gross oxygenic productivity (P) in creek-bank sediments. Dominance represented as the proportion (from 0.0 to 1.0) of taxon-specific pigment over total pigment abundance (B_5). Peridinin (found in only two cores) dominance not shown. All productivities measured at 72 µE m^{-2} s^{-1} (surface) irradiance.

Figure 4.5. Functional group diversity effects on the depth in the sediment of highest gross productivity for sediment communities collected from creek-banks (summer 2002).

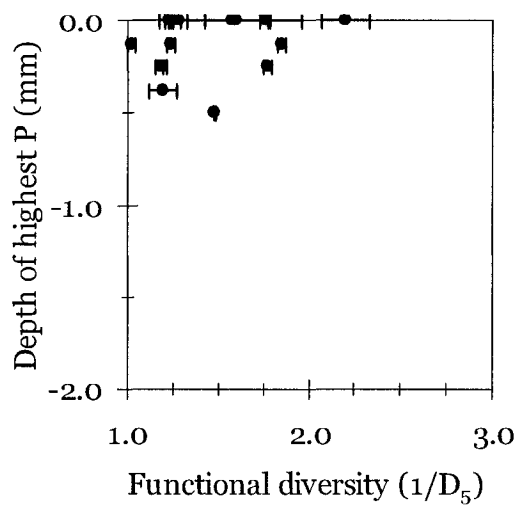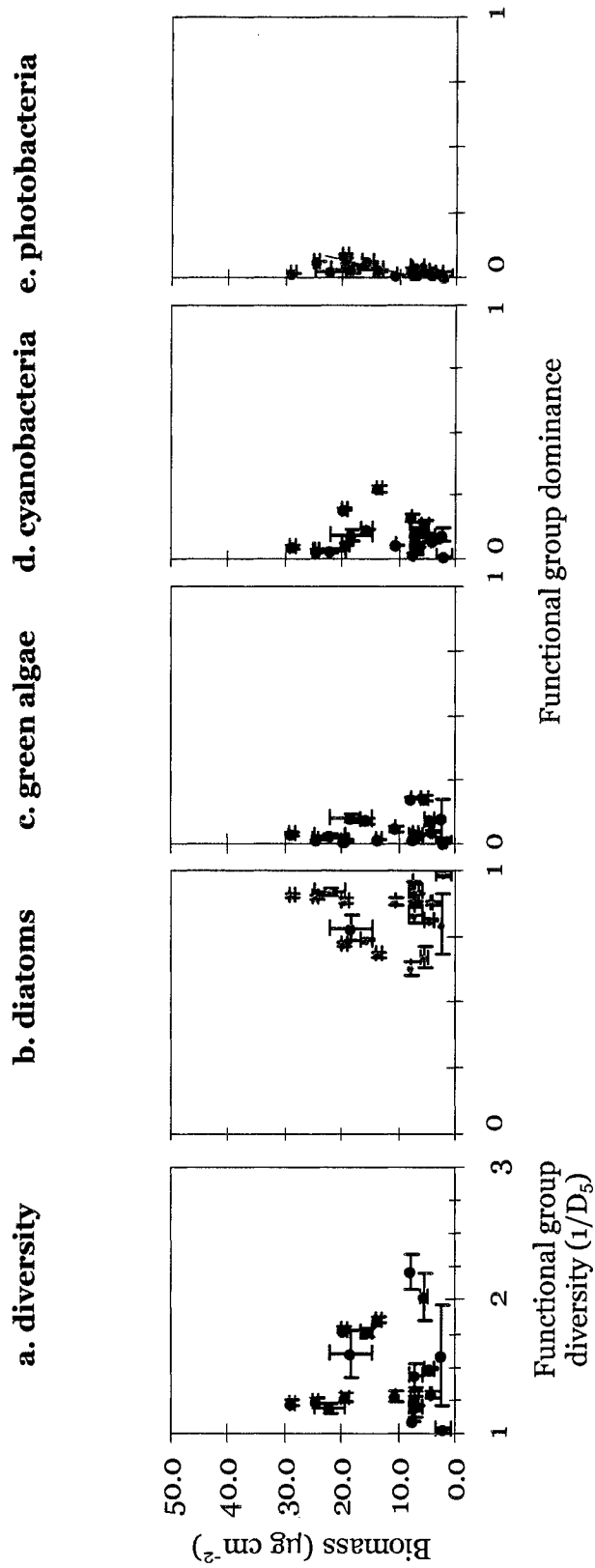**Figure 4.6.** Association between (a) mean (±1 S.E.) diversity and (b-e) mean (±1 S.E.) functional group dominance (b-e) and mean (±1 S.E.) microphototroph biomass (B5) in creek-bank sediments.

Functional group diversity had no apparent influence on gross productivity or on biomass, but several environmental variables were tested to quantify their relationship to these measures of production. Light transmission through plant canopies, sediment organic matter content, and sediment sandiness failed to explain a significant proportion of variation in gross photosynthesis (all $p>0.5$). Furthermore, light transmission and sediment sandiness also did not explain variability in microphytobenthic biomass (both $p>0.1$), but organic matter was strongly associated with the biomass (B_5) of microphototrophs ($p<0.0001$).

Diversity-production in natural halophyte salt marsh. Sediment communities collected during summer 2004 were obtained from salt marsh plant communities containing between 1 and 7 or 8 vascular plant species. Generally, plant assemblages tended to possess moderate canopy cover. Sediment pore water salinities (mean $\pm$ 1S.D. = 72.2 $\pm$ 44.8, n=32) and surface temperatures were also rather high, reflecting summer-time desiccation stress at low tide. Microscopic observation of sediment from a subset of live cores indicated the presence of cyanobacteria (filamentous and coccoid morphologies), diatoms (solitary pennates and chain-formers), green algae, motile granule-laden filaments (possibly phototrophic bacteria) and other unidentified pigmented organisms (Figure 4.3). Coccoid cyanobacteria such as *Chroococcus/Gloeocapsa*, pennate diatoms, and filamentous cyanobacteria (Oscillatoriales) were relatively common (see Appendix 1).

Pigment analysis of sediments from these halophyte marsh sediments demonstrated that communities were often dominated by the pigment fucoxanthin, but concentrations of bacteriochlorophyll a occasionally comprised greater than 40% of the total abundance of targeted pigments (B_4), perhaps due to some extent to extended incubation times in the lab under more or less anoxic conditions. Lutein

(derived from green algae and plant detritus) was present in only minor quantities in these assemblages. Functional group diversity ($1/D_4$) in the communities varied from 1.1 to 2.3 (mean ± S.E. = 1.64 ± 0.07). Biomass (as B_4) varied between 2.1 and 31.6 μg cm^{-2} whereas chlorophyll a concentrations from 2.2 to 27.7 μg cm^{-2} were observed. For the subset of 16 cores for which reliable gross oxygenic photosynthesis was obtained, chlorophyll a-normalized, depth-integrated production varied from 0.0005 to 0.0072 μg O_2 μg chl a^{-1} s^{-1}.

Like creek-bank communities, integrated gross oxygenic productivity was unrelated to functional group diversity in these marsh microphototroph assemblages (Figure 4.7, p>0.8). Furthermore, fucoxanthin dominance, zeaxanthin dominance, lutein dominance, and bacteriochlorophyll a dominance all failed to predict gross productivity (p>0.6, >0.6, >0.3 and >0.6 respectively). Functional group diversity was also unrelated to the overall depth of the production zone (greatest depth at which positive production was observed; p>0.5; Figure 4.8a) and was not predictive of the depth in the sediment where highest production rates were observed (p>0.3; Figure 4.8b).

Microproducer biomass was positively related to functional diversity with highest total pigment concentrations (B_4) seen in communities of moderately-high diversity (r=0.40, p=0.027; Figure 4.9). This diversity influence on biomass was not observed, however, when microproducer abundance was estimated by sediment chlorophyll a concentration (p=0.14). Fucoxanthin and zeaxanthin dominance were negatively related to community biomass (r=-0.42, p=0.02 and r=-0.38, p=0.04 respectively) but bacteriochlorophyll a dominance (r=0.46, p=0.009) and green algal dominance (r=0.36, p=0.05) were positively associated with phototroph standing stocks.
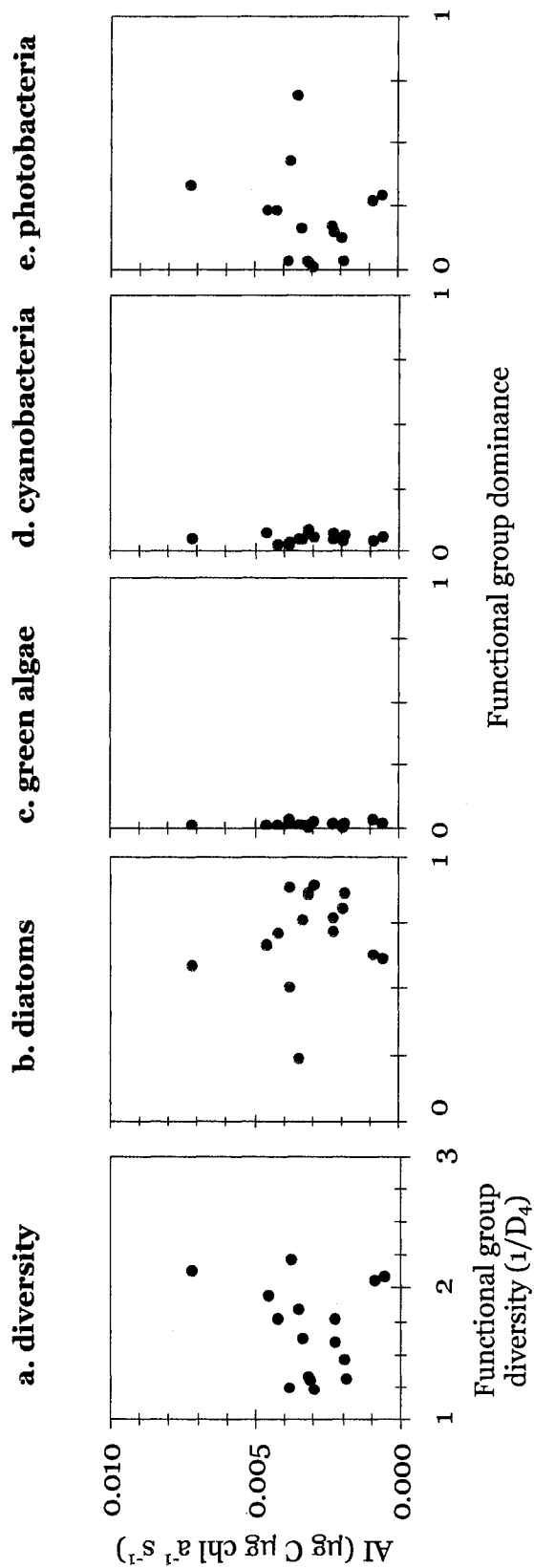
Figure 4.7. The effects of (a) mean (±1 S.E.) diversity and (b-e) mean (±1 S.E.) functional group dominance on mean (± 1S.E.) gross oxygenic productivity (P) in sediments from salt marsh. Dominance represented as proportion (from 0.0 to 1.0) of taxon-specific pigment over total pigment abundance (B$_4$). All productivities measured at 61 µE m^{-2} s^{-1} (surface) irradiance.

Figure 4.8. Functional group diversity effects on (a) the depth in the sediment column of highest gross productivity and (b) the maximum depth to which observable gross productivity was detected.

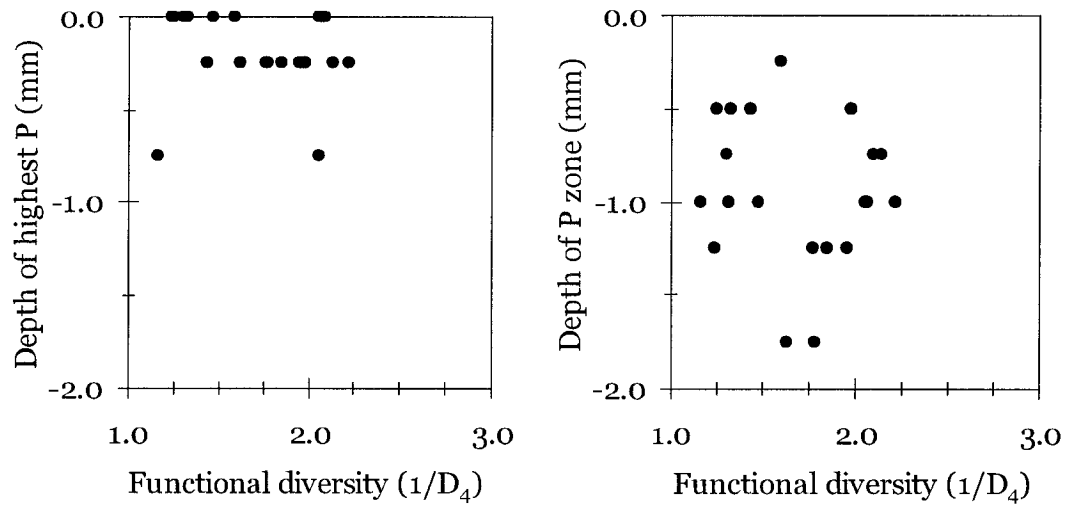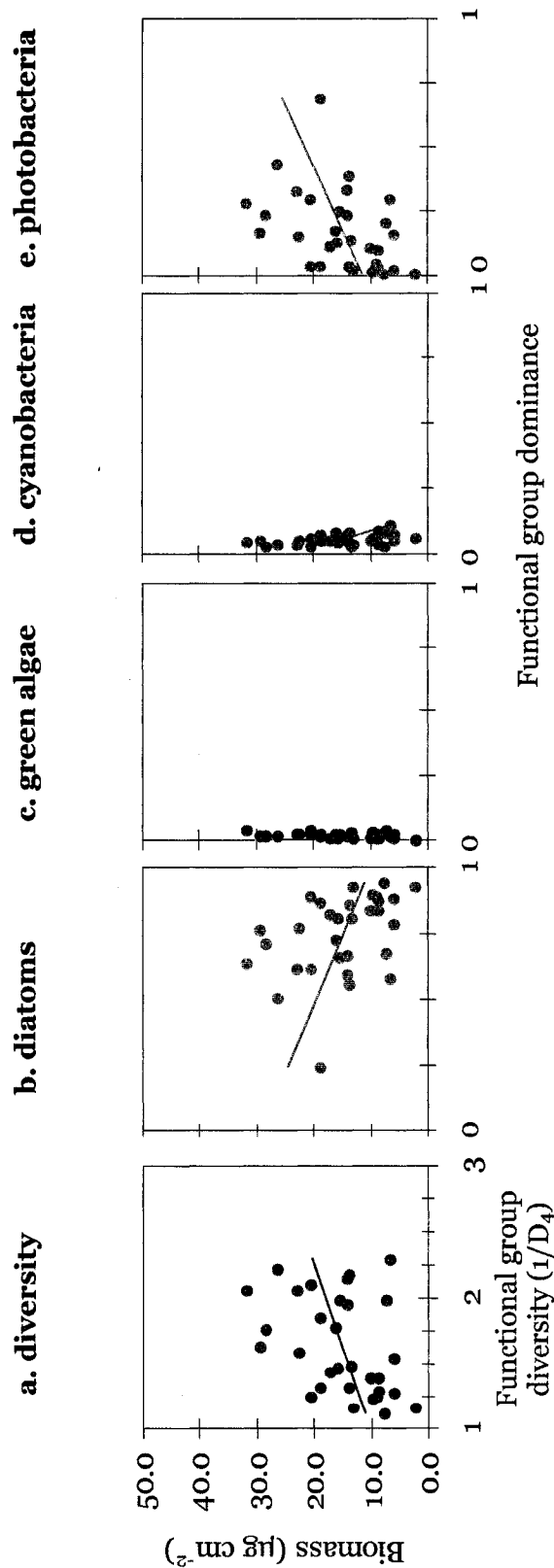**Figure 4.9.** Association between (a) mean (±1 S.E.) diversity and (b-e) mean (±1 S.E.) functional group dominance and mean (±1 S.E.) microphototroph biomass (B_4) in salt marsh sediments.

Functional group richness and productivity in microcosms. Cultures composed of green algae were about 7 and 15 times as productive as diatom and cyanobacteria cultures respectively, (Figure 4.10a), but due to high variability and/or low replication of treatments, no significant effect of composition on productivity rates were observed ($F_{3,10}=2.0$, $p=0.18$). Additionally pooled mono-specific cultures (n=12) were equally as productive as cultures composed of three functional groups (p>0.6; Figure 4.10b). *A posteriori* comparisons showed that more diverse assemblages had significantly higher productivity than cultures consisting of cyanobacteria grown alone ($F_{1,11}=5.6$, $p=0.04$) but had similar rates to diatom cultures ($F_{1,9}=0.6$, p>0.4). In laboratory microcosms, functional group richness did not explain variability in the depth of greatest production ($F_{1,19}=1.4$, p>0.2; Figure 4.10c).

Diversity-biomass relationships in restored versus natural wetland. Sediment communities from *Spartina* and mudflat habitats from a restored wetland and nearby natural habitat in Tijuana Estuary (see Chapter III) were analyzed for variation in diversity-biomass relationships over the early period of restoration site development (0.2 to 3.2 yrs). On most dates, diversity and biomass were not correlated in either mudflat or in *Spartina foliosa* salt marsh, however, a few positive relationships were seen in natural wetland and a few negative relationships were observed in restored sediment communities (Table 4.1). Biomass-diversity relationships were also investigated for a large set of microproducer communities collected from Mission Bay during March 2002, but no relationships between biomass and diversity were observed in any habitat. In the few cases where significant differences between restored and natural correlation coefficient were observed, diversity-biomass relationships tended to be more negative in restored wetland than in natural habitat.
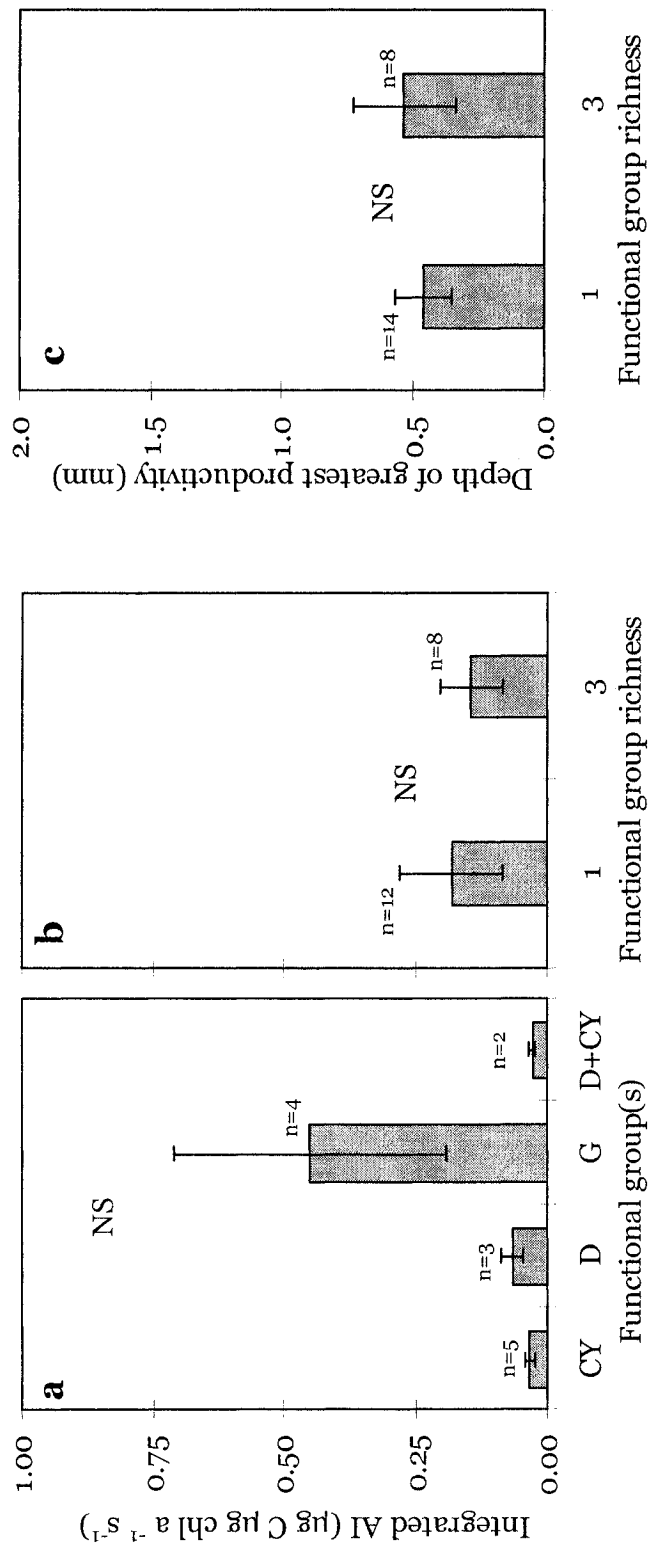
Figure 4.10. Gross productivity in laboratory microcosms. (a) Photosynthetic rates (as AI) for each single functional group treatment and for two diatom+cyanobacteria (D+CY) replicates. Depth integrated production was scaled by chlorophyll a concentration (in µg cm⁻³). (b) Functional group richness effects on production. Single functional group treatments are pooled. (c) Functional group effects on the depth (below the substrate surface) of highest productivity. All productivities measured at 200 µE m⁻² s⁻¹ (surface) irradiance. NS= not significant.

Table 4.1. Biomass (B_3 or B_4) and diversity ($1/D_3$ or $1/D_4$) relationships in natural and restored wetlands in Mission Bay (MBAY) and Tijuana Estuary (TIES). Sediment communities were separated according to sampling date and habitat. Differences between natural and restored wetland relationships (χ^2 test on correlation coefficients, r) are shown at far right.

Site sampled	Date	Habitat	Comparison	REST age (yr)	NATURAL r	p	n	RESTORED r	p	n	REST-NATURAL comparison (χ^2 test)
TIES	Apr-00	mudflat	B_3 vs. $1/D_3$	0.2	-0.43	>0.3	6	-0.65	0.17	6	χ^2=0.1; p>0.7
	Apr-00	*Spartina* marsh	B_3 vs. $1/D_3$	0.2	0.94	**0.005**	6	-0.57	>0.2	6	χ^2=8.9; **p=0.003**
	Apr-01	mudflat	B_3 vs. $1/D_3$	1.2	-0.41	>0.4	6	-0.16	>0.6	9	χ^2=0.2; p>0.7
	Apr-01	*Spartina* marsh	B_3 vs. $1/D_3$	1.2	0.50	0.12	11	0.01	>0.9	11	χ^2=1.3; p>0.2
	Sep-01	mudflat	B_3 vs. $1/D_3$	1.7	0.93	**0.001**	8	0.44	>0.3	7	χ^2=2.9; **p=0.09**
	Sep-01	*Spartina* marsh	B_3 vs. $1/D_3$	1.7	-0.01	>0.9	8	-0.98	**0.004**	5	χ^2=7.4; **p=0.007**
	Apr-02	mudflat	B_4 vs. $1/D_4$	2.2	0.26	>0.5	9	-0.27	>0.6	5	χ^2=0.4; p>0.5
	Apr-02	*Spartina* marsh	B_4 vs. $1/D_4$	2.2	0.10	>0.8	6	-0.43	0.40	6	χ^2=0.5; p=0.5
	Apr-03	*Spartina* marsh	B_3 vs. $1/D_3$	3.2	-0.19	>0.6	8	-0.78	**0.02**	8	χ^2=1.8; p=0.18
MBAY	Mar-02	mudflat/creeks	B_4 vs. $1/D_4$	6.3	-0.21	>0.4	16	0.18	>0.6	8	χ^2=0.7; p>0.3
	Mar-02	*Spartina* marsh	B_4 vs. $1/D_4$	6.3	0.15	>0.7	8	-0.56	0.15	8	χ^2=1.8; p=0.18
	Mar-02	halophyte marsh	B_4 vs. $1/D_4$	6.3	-0.51	0.19	8	-0.46	>0.2	8	χ^2<0.1; p>0.9

Biomass-diversity relationships

Discussion

The growing body of studies examining diversity-function relationships in marine ecosystems provides mixed evidence for a positive effect of high biological diversity on ecosystem functions. Among marine studies that examine primary production in the context of variable species richness, for example, there is evidence for enhancement of productivity at higher diversity (Agard et al. 1996, Callaway et al. 2003) but also support for the notion that richness has little impact on production (Paine 2002). The present work also provides mixed results, with evidence that the nature of diversity-productivity relationships may depend on the method used to quantify production.

Lack of a consistent positive diversity influence on primary production in marine ecosystems is similar to the broad picture gained from terrestrial plant studies (Waide et al. 1999), but does contrast markedly with results from several intensive grassland manipulations conducted on small spatial scales where there is evidence for both the promotion of greater biomass by higher plant diversity and niche complementarity as a mechanistic explanation for those relationships (Tilman et al. 2001, Knops et al. 2004, Spehn et al. 2005, van Ruijven and Berendse 2005). Indeed, in several of those experiments, the strength of diversity effects on function actually appears to have increased from year to year (Tilman et al. 2001, Spehn et al. 2005, van Ruijven and Berendse 2005). Nevertheless, even in temperate grasslands, high diversity does not necessarily enhance all ecosystem functions (Spehn et al. 2005), and its effects can be overshadowed by the influence of additional factors (e.g., site, nutrient levels) on functional attributes (Fridley 2002). Species composition may be as equally influential as diversity on ecosystem processes (Symstad et al. 1998).

As a whole, there is little research that addresses the question of diversity-productivity relationships among microproducers (but see Naeem and Li 1997, for example) and there are few studies that attempt to make estimates of gross photosynthetic production in addition to measures of biomass accumulation (however, Agard et al. 1996 measured net production in phytoplankton communities). While biomass-based studies can integrate the effects of diversity on production over longer periods of time (assuming grazing and respiration losses do not confound diversity treatments), estimates of gross productivity provide information on the physiological condition of producers on short time scales. The present study appears to be the first to address the question of how diversity and production are related in marine primary producers using simultaneous measures of gross primary production and standing stocks.

Microproducer diversity and gross productivity. Gross photosynthesis measurements in `natural wetland assemblages (both salt marsh and creek-bank habitat) suggested that depth-integrated oxygenic production was largely un-influenced by low ($1/D_4 \approx 1$) to moderate ($1/D_4 \approx 2$) functional diversity (Figures 4.4, 4.7). There was weak evidence pointing towards a negative association between cyanobacterial dominance and photosynthetic rates (creek-bank sediments only) but the relative abundances of other functional groups were apparently not related to short-term productivity in field-collected communities.

Pigment concentrations in the sediments at the sites investigated here suggested that communities were composed primarily of diatoms. Thus based on their overall dominance, it is probable that the majority of non-vascular plant carbon fixation in many coastal wetland ecosystems is accomplished by epi-benthic diatoms. The vertical migration of many pennate taxa (Underwood et al. 2005), relatively high

sulfide and salinity tolerance of diatoms (Admiraal 1984, Clavero et al. 2000), and possibly enhanced nutrient uptake rates relative to other functional groups may confer a competitive advantage over less motile cyanobacteria and the non-motile green algae.

Like natural communities, there was little diversity influence on production in laboratory-constructed cultures, although higher functional richness lead to higher photosynthetic rates relative to monospecific communities composed of only cyanobacteria. The presence or absence of green algae may, in part, contribute to variation in photosynthetic rates, but statistically significant differences were elusive because of low replication. In addition, small changes (at the sub-millimeter scale) in the proximity of green algal thalli to the site of microelectrode work may have contributed to substantial variation in green algal photosynthetic rates. For example, a few additional photosynthetic measurements made on the surface and interior of green algal clusters revealed photosynthetic rates an order of magnitude greater than any observed in any other cultures, regardless of functional group richness (data not shown). Finally two additional cautions should be expressed with respect to green algal measurements: (a) green algal biomass was locally concentrated (near the site of production work) because the organisms used were small discreet thalli but was low on a per-core basis, potentially inflating estimates of chlorophyll a-normalized gross photosynthesis, and (b) I was unable to obtain completely pure green algal treatments because of some cyanobacterial contamination. Given these difficulties, the possibility of an effect of taxon-identity on gross photosynthesis in the lab that should be further investigated in more comprehensive experiments.

The cultured organisms used in the laboratory manipulation may be relatively common in wetland sediments from Mission Bay, but they represent only a subset of

total microphototrophs present at the site. For example, at least 18 morphospecies of cyanobacteria and three genera of green algae appear to be present in Mission Bay sediments (Appendix 1), and dozens of diatom species are known from the nearby Tijuana Estuary (Zedler 1982) and from Mugu Lagoon in southern California (Wilson 1980). It is unknown how many of these species might eventually be brought into successful culture, but further diversity-function work might focus on testing productivity responses to changes in diversity with species of different morphology and physiology and under multiple light, nutrient, and disturbance conditions.

Microproducer diversity and biomass. In contrast with rates of gross production, biomass was mildly related to functional diversity within mixed halophyte salt marsh sediments (Figure 4.9). In this habitat, significant biomass-dominance relationships were observed with pigments from diatoms and cyanobacteria (negative) and green algae and phototrophic bacteria (positive). A switch from dominance by diatoms to increased representation of anoxygenic producers and green algae in communities was associated with greater standing stocks of microphototrophs. In unvegetated creek-bank habitat, bacteriochlorophyll a dominance was again positively associated with community biomass, but the relative abundances of no other pigments appeared to be associated with variation in standing stocks (Figure 4.6).

Biomass is regarded by some ecologists as a poor measure of photosynthetic production (Leith 1975), but it is commonly measured in both marine and terrestrial habitats and has often been employed as a surrogate for ecosystem productivity in diversity-function manipulations (Hector et al. 1999, Reich et al. 2004). In many plant communities, including wetland microproducer assemblages, biomass may be influenced by nutrient availability, grazing pressure, and more generally, by

successional trajectories (see Leith 1975) and thus represent a time-integrated amalgam of multiple abiotic and biotic processes. Several non-causal factors may concurrently drive variation in both diversity and standing stocks of microproducers, producing some of the significant relationships seen in this study: (1) selective grazing by wetland consumers that simultaneously reduces community diversity and biomass and (2) increased stress that eliminates more sensitive taxa and reduces the productivity of remaining community members.

Alternatively, there are several potential mechanisms that could lead to a more *direct* control of microproducer standing stocks by community diversity. First, more diverse microbial assemblages may be more difficult to disrupt by invertebrate grazers or may be more resistant to hydrodynamically-mediated erosion because of the structural complexity of mats that contain filamentous cyanobacteria. Second, positive diversity effects on biomass may arise because more diverse communities are better able to acquire abiotic resources (light or nutrients) which in turn lead to greater carbon fixation per unit area of sediment. For example, in localized cases of N, P, Si, or trace elemental limitation, higher functional diversity might positively affect the efficiency of nutrient capture or turnover time of nutrient cycles such that communities attain higher community biomass. Specifically, diatoms and green algae may benefit in conditions of low concentrations of bio-available nitrogen by cyanobacteria or phototrophic bacteria diazotrophy and subsequent N leakage. Indeed, other researchers suggest that possible phototroph-heterotroph interactions and anoxygenic-oxygenic associations in benthic microbial mats might possibly enhance the efficiency of carbon and nitrogen dynamics (Paerl et al. 1993, Pinckney and Paerl 1997).

Finally, a niche-filling mechanism may account for the positive association

between diversity and biomass (Figure 4.11). In this case, the sediment column can be thought to be partitioned into three discreet vertical zones: an epi-benthic zone at the sediment-water interface, an upper oxic sediment layer (probably usually no more that a few mm thick), and a sub-surface anoxic (but photic) layer of sulfide-rich sediments. Green algae, diatoms+cyanobacteria, and phototrophic bacteria are the principle constituents of each of these zones. As these niches are occupied by their respective taxa, total community biomass (on a per-area basis) would be expected to increase concurrently with functional diversity. Although competition for light is also expected to some extent, longer wavelengths can penetrate to deeper layers (Pierson et al. 1990) for capture by bacteriochlorophyll-containing anoxygenic phototrophs (Stal 2000).

This niche-based mechanism is similar to the concept of niche complementarity which suggests that production is enhanced when more diverse communities better utilize habitat resources (Tilman and Lehman 2001). In this case, space and/or light may be the limiting resources. A niche-based explanation of diversity-productivity relationships was invoked by Carlander (1955) a half century ago who noted that in freshwater lakes, filling of available niches in an ecosystem could simultaneously lead to higher diversity and to greater standing stocks of fishes.

A few lines of evidence support these ideas. First, as already mentioned, bacteriochlorophyll a dominance and total community biomass were strongly associated in both kinds of habitat studied (Figures 4.6, 4.9). Moreover, within mixed halophyte salt marsh and creek-banks, there were tight relationships between absolute sediment concentrations of bacteriochlorophyll a and total community biomass (both $r > 0.75$, both $p < 0.0001$, n=31 for marsh, n=20 for creek-banks; data not shown) and positive relationships between sediment bacteriochlorophyll a
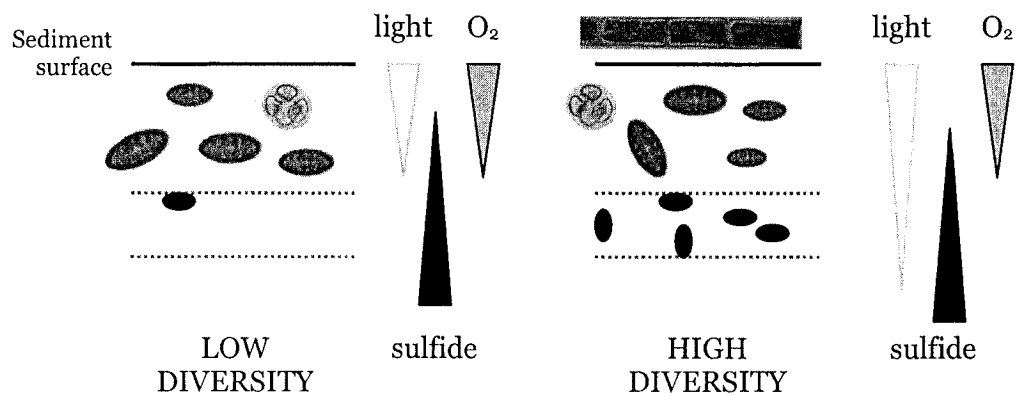
Figure 4.11. A hypothetical niche-filling model that could simultaneously lead to high diversity and high standing stocks in sediment benthic microproducers. The low diversity community is dominated by diatoms and contains only a few cyanobacteria and phototrophic bacteria. A higher diversity assemblage contains an epi-benthic layer of green algae and more phototrophic bacteria in addition to the typical oxygenic phototrophs in the upper sediment layer. Niche availability may be mediated relative concentrations of oxygen and sulfide and by light attenuation.

benthic concentrations and sediment fucoxanthin concentrations (r=0.38, p=0.034, n=31 in marsh, r=0.75, p=0.0002, n=20 in creek-banks; data not shown) implying lack of competition between these major two functional groups. Finally, epi-benthic green algae

Environmentally-mediated variation in diversity-productivity relationships. Diversity-function relationships may change qualitatively under different environmental regimes or along gradients of stress and disturbance (Mulder et al. 2001, Cardinale et al. 2004). In fact, several models suggest that a range of diversity-function relationships are possible in nature (Cardinale et al. 2000). The challenge for ecologists is to predict and test the abiotic conditions (e.g., high stress, Mulder et al. 2001), the spatial scales (Cardinale et al. 2000), and time scales over which diversity will have a consistently positive influence on ecosystem processes. While simultaneous controlled manipulation of diversity and environmental factors was not conducted in this study, data collected from mensurative work on sediment microphototrophs (Chapters II, III) allowed a limited test of biomass-diversity relationships between natural (undisturbed) and restored (disturbed) wetlands from two southern California sites. In temperate wetlands, restored sites can be viewed as environments of higher stress because they often contain a lower proportion of organic matter (Edwards and Proffitt 2003) and a lower proportion of fine-grained sediments (Campbell et al. 2002) than corresponding natural habitat. At restored sites, differences in salinity, plant cover, and invertebrate (e.g., grazer) biomass can also exist between disturbed and natural habitat (Levin and Talley 2002, Chapter II).

Two alternative models predict the outcome of diversity-biomass relationships along gradients of disturbance or stress. First, if positive interactions play an

important role in organism-organism interactions in highly disturbed communities, more diverse assemblages may better cope with abiotic stress than less diverse communities, and hence be more productive. On the other hand, because disturbed habitat may possess a distinct set of environmental conditions that are favored by only a few specialist taxa, production would be expected to be higher under low diversity assemblages (Mayer and Galatowitsch 2001).

The mensurative data analyzed here suggested that a variety of diversity-biomass relationships exist in sediment microfloras (Table 4.1). Such wide heterogeneity in the kinds of relationships seen was probably driven in part by small to moderate sample sizes. However, a few analyses suggested moderate to strong differences in diversity-biomass relationships between restored and natural habitat. When differences existed, natural marsh communities tended to show positive or neutral biodiversity-biomass relationships and restored wetlands tended to show more negative associations. Although the mechanistic explanation for these results is unknown, it is possible that restored habitat has a tendency to possess environmental conditions that promote low diversity but high biomass assemblages (see Mayer and Galatowitsch 2001). In addition, since so many diversity-biomass relationships were non-significant, it is also possible that the differences in abiotic features of restored and natural wetlands may not be sufficiently large to generate qualitatively different mechanisms of co-existence in microproducers. Because environmental clines may be steep in marine intertidal habitats (with more intense desiccation, higher temperatures and elevated salinities at elevations seldom covered by tides), there may be seasons or microhabitats wherein the effects of diversity on function may be more pronounced.

Conclusions. Coastal marine wetlands are transitional habitats which are often characterized by relatively low levels of diversity (Levin et al. 2001) including only a few major functional groups of photosynthetic algae and bacteria. Diatoms dominate floras generally, and probably account for the bulk of marine wetland productivity. However, because physiological and metabolic differences among taxonomic groups are present in sediment floras, there may be little support for high functional redundancy in wetland microproducers (see Zedler et al. 2001). Interestingly, it may be because other functional groups often do not occur in great abundance that natural levels of variation in diversity appear to have little overall effect on gross productivity or standing stocks. At least two hypotheses deserve further attention: (a) green algae may be superior oxygenic producers under certain environmental conditions, and (b) anoxygenic phototrophs may concurrently enhance diversity and productivity in localized patches of high organic content as they fill a sediment niche unavailable to eukaryotic producers. Changes in microphototroph functional diversity may drive rates of other wetland processes (e.g., nutrient cycling, nitrogen fixation) and this should be further explored via field research and microcosm experiments. I suggest, like Zedler et al. (2001), that physiological specialization and specific microhabitat affinity by different functional groups imply the need for all major taxa to ensure the maintenance of normal levels of productivity in wetland sediments.

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Chapter V.

Conclusions: integration of composition and diversity into conceptual models of

microphytobenthic ecology

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Introduction

This dissertation has addressed several ecological aspects of microproducer diversity and composition in sediments from marine wetlands. I have explored community compositional changes during wetland development, the distribution of major microproducer taxa at different spatial scales, and the association between diversity, composition, and primary productivity. Here I briefly review the major findings of each of the major dissertation chapters and touch on additional conclusions that emerge from the study of these organisms. I comment on the implications of these findings for coastal wetland management strategies and suggest several future directions to address many of the remaining unanswered questions about wetland microphytobenthic community dynamics.

Spatial and temporal variation in composition

Microphytobenthic composition and diversity at the functional group level can vary at several distinct spatial scales. Previous research suggests, for example, that cross-intertidal differences in composition and diversity may be particularly prevalent (e.g., Javor and Castenholz 1981, Rothrock and Garcia-Pichel 2002) with higher microproducer diversity often found at higher elevations. The work conducted here expands upon our knowledge of three additional scales of variability in microproducer biodiversity and composition: (1) variation between visually distinct patches on the sediment surface (see also Paterson et al. 1998), (2) differences between the major kinds of habitats present in coastal marine wetlands (see also Zedler 1982), and (3) heterogeneity between restored and natural ecosystems.

Patches. In Chapter III I elucidated patch-specific spatial patterns of pigment composition in a large restoration site. Visually distinct patches (as small as <1 m²) were characterized by significant differences in microphototroph abundance, functional diversity, and the relative abundance of diatoms and cyanobacteria (Figures 2.2-2.6). Particular communities were associated with small scale features of the sediment surface such as shallow depressions (pools), overlying macroalgal rafts, and emergent drier intertidal sediments. These data imply that minor features on the sediment surface can have substantial impacts on the community structure of microphytobenthos.

Habitats. Habitat-level variation in community composition was also evident throughout this study. Specifically, there was evidence for (1) differences in measures of community structure, and (2) differences in the rate of ecosystem development. In the Mission Bay communities sampled in spring 2002 (Chapter II), for example, I found that the two kinds of vegetated marsh investigated were characterized by higher functional diversities and by relatively more cyanobacteria than either intertidal mudflats or unvegetated creek-bank sediments. Higher functional diversity in the vegetated habitats was likely largely driven by an increased abundance of anoxygenic bacterial producers (Figure 2.2) and cyanobacteria (Figure 2.5). However, at this place and time, there was little evidence for any meaningful differences in microproducer communities between *Spartina* marsh and mixed halophyte marsh within the natural wetland. This differs from the species-level results of Zedler (1982) who found compositional differences associated with different kinds of vascular plant canopies in Tijuana Estuary. Perhaps compositional differences between different vascular plant canopies are expressed more at the species level than at the functional group level.

Habitat-level differences in natural communities present in Mission Bay suggest that photosynthetic prokaryotes have a preference for lower light conditions (see also Sullivan 1976, Stal 2000), but interestingly this conclusion is not supported by the data from Tijuana Estuary. Here, cyanobacteria were as an important a component of natural marsh mudflats as they were in natural *Spartina foliosa* habitat (compare Figures 2.8a and 2.8b) and functional diversity was just as high in mudflats as in vegetated marsh. In recent plant canopy removal and sediment shading manipulations in Mission Bay salt marsh, Whitcraft (pers. comm.) also failed to find any changes in zeaxanthin/fucoxanthin ratios after 3-6 months in response to altered light regimes. Thus additional experimental manipulations of light intensity and observations of other sites are needed to better understand community-level responses to this important variable.

Data from Mission Bay also suggest that organic matter may play an important role in community composition. Organic-rich sediments were more likely to have higher bacteriochlorophyll a/chlorophyll a ratios (communities more enriched in anoxygenic producers relative to oxygenic producers) and may be more likely to have relative increases in cyanobacteria (relationship significant only for natural marsh). Since organic matter may also be tightly linked to nutrient (N, P) concentrations, multi-factor enrichment experiments are needed to determine if higher N, P, POC , DOC or all of the above tend to shift community composition towards greater dominance by prokaryotic phototrophs. Research on other organisms in restored wetlands showed that higher organic matter may also be tied to greater invertebrate densities (Craft 2000) and healthier vascular plants (McCray 2001), so organic matter development may have wide ecosystem-level effects in salt marsh development.

Habitat-level differences were also observed in the tempo of wetland development. At the Friendship Marsh in Tijuana Estuary, there was rapid recovery of microproducer composition and diversity in *Spartina foliosa* marsh but slower (>1.5 yr) development of cyanobacterial floras in restored mudflats. Low organic matter and/or higher rates of grazing in restored mudflat may have been causes of delayed recovery in cyanobacteria.

Restored versus natural sites. My results also suggest that there can be substantial variation between restored and natural wetland habitats. Pigment analyses from the small restoration site at Mission Bay (age=6.3 yr) suggested that restored *Spartina* marsh was very similar to natural *S. foliosa* habitat in terms of microproducer biomass, composition and functional diversity. However, natural and restored creek-banks in Mission Bay were only partially similar, with the latter enriched in bacteriochlorophyll a. Additionally, very pronounced differences in community structure were observed between restored and natural mixed halophyte salt marsh. Restored halophyte marsh consistently hosted lower oxygenic and anoxygenic phototroph biomass and lower functional group diversity than sediments in natural habitat. Because mixed halophyte marsh is the most common intertidal habitat next to unvegetated mudflats in Mission Bay, it should consequently be an important focus of wetland restoration work. More generally, the habitat-level comparisons of microproducer communities in natural and restored wetlands in Chapter II suggest that each kind of habitat present in mitigation work needs to be studied separately to evaluate the success of restoration efforts.

These studies of spatio-temporal differences in microproducer communities also provide information useful for future engineering of coastal wetland mitigation projects. Because different microphytobenthic groups may be favored by different

kinds of conditions in wetland sediments, it may be important to provide structural heterogeneity in habitat mitigation work to ensure the success of all major groups. This would also lead to the greatest chance that all of the varied functions normally carried out by microphytobenthic taxa (e.g., production, nitrogen fixation, etc.) are present in restored habitats. The data here suggest that restored or created wetland sites should include (a) both planted and unplanted habitats to ensure both diatom and diatom+bacterial producer communities, (b) shallow intertidal pools to promote cyanobacterial communities, and (c) at least some high organic matter sediments for the development of anoxygenic photobacteria and cyanobacteria.

The nature of microproducer assemblages in southern California wetlands

Analysis of photopigments from numerous microphytobenthic communities from both Tijuana Estuary and Mission Bay (in a variety of wetland habitats and across a variety of seasons and years) establish several important patterns about community structure: (1) diatoms are consistently the dominant group in wetland sediments, (2) functional diversity is often low, and (3) evenness, not richness, largely drives diversity patterns. These major points are discussed further below.

Sediment fucoxanthin concentrations suggested diatom dominance across all sites, seasons and habitats. Many wetland studies from various temperate salt marshes and mudflats around the world also show overall diatom dominance in coastal sedimentary habitats (Underwood 1994, Peletier 1996, Sundbäck et al. 1996a, Brotas and Plante-Cuny 1998, Thornton et al. 2002, Zheng et al. 2004). Indeed, cyanobacteria-dominated sedimentary communities may be relatively rare in coastal

marine environments, perhaps limited to habitats supporting warm hypersaline

shallow water (Pinckney and Paerl 1997, Nübel et al. 1999) or low grazing pressure

(Stal 2000). For example, a wide variety of anecdotal evidence (such as the

commonly observed prevalence of cyanobacteria in the summer) and experimental

data (Waterman et al. 1999), suggest that greater cyanobacteria dominance may be

linked to higher temperatures.

Diatom dominance resulted in low functional diversities in the majority of

sediments collected. Diversity showed a highly skewed distribution towards the

lowest possible numbers (Figure 5.1). However, despite overall diatom dominance,

pigment analysis revealed that almost all communities possess at least some pigments

from the other principle taxonomic groups: cyanobacteria and anoxygenic

phototrophic bacteria. Green algal representation was not quite as ubiquitous across

communities, but lutein could still be found in the majority of sediment assemblages.

These observations suggest that essentially all intertidal wetland sediments at these

sites could contain at least some of the major microphytobenthic functional groups

(although pigment analysis alone cannot establish whether living or dead organisms

contributed to sediment pigment concentrations) and show that variation in

functional group diversity is driven by changes in functional group evenness much

more than richness. This furthermore may imply rapid rates of dispersal of all major

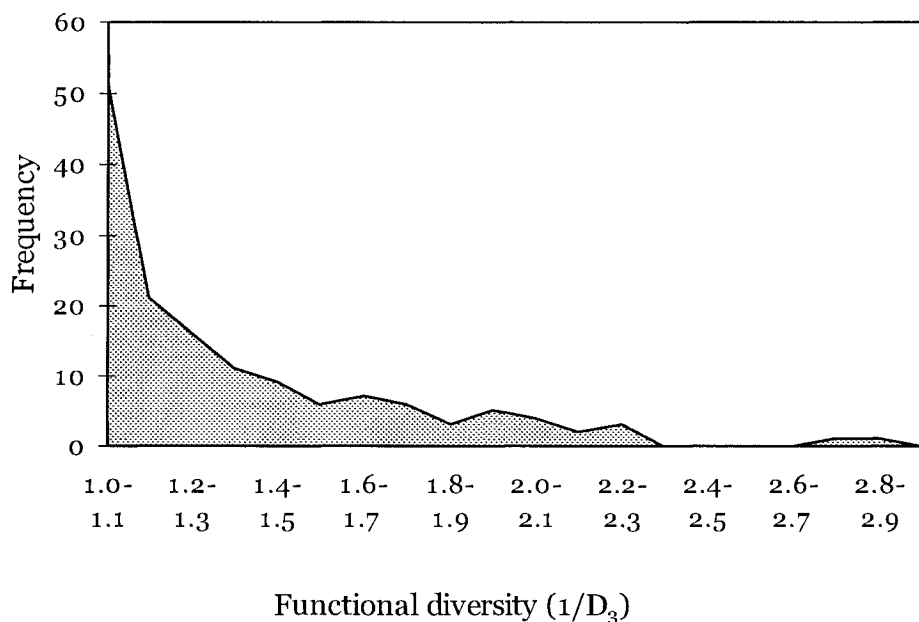functional groups throughout wetland habitats.

Functional diversity (1/D$_3$)

Figure 5.1. Frequency distribution of the functional diversity of microphytobenthic communities collected from restored and natural Tijuana Estuary sediments (Chapter III, n=146).

As noted, changes in evenness were the main cause of increases in functional diversity. Interestingly, increases in relative abundance were distributed fairly evenly across the other three major functional groups (Figure 2.8). Green algae were usually rare at the (small) scale over which sampling was conducted and may have had an underestimated impact on functional diversity. Additionally, the identity of which functional group contributed most to higher diversity may have differed between sites: in Mission Bay anoxygenic photobacteria and cyanobacteria may have been slightly more important, at Tijuana green algae may have been slightly more common (compare Figures 2.8 c and d). For practical purposes, the ratio of cyanobacteria to diatoms (zeax/fuco) can explain much of the variation in microproducer functional diversity (Figure 5.2).
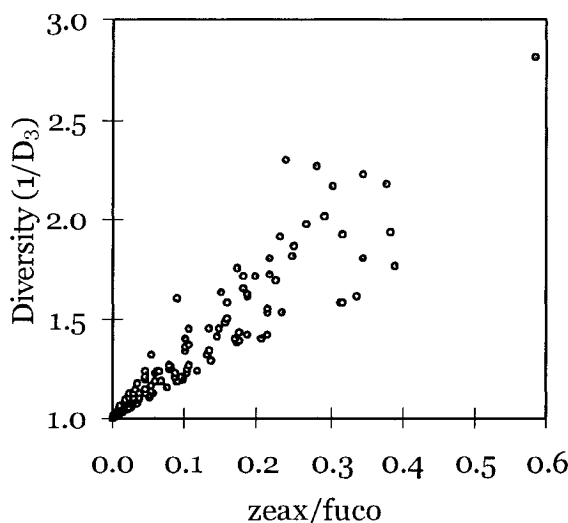
Figure 5.2. The relationship between cyanobacteria to diatom ratios (zeax/fuco) and functional group diversity in pooled Tijuana Estuary sediment communities (natural and restored communities in Chapter II). Diversity ($1/D_3$) was estimated based only on the abundance of zeaxanthin, fucoxanthin, and lutein.

Other taxonomic groups (*Vaucheria* and euglenoids) were also confirmed members of sediment communities (see Appendix 1), but these organisms were not consistently present in collected samples. Confirmation of euglenoid presence by pigment detection would be difficult since they share all their major pigments with other taxa common to wetlands (e.g., diatoxanthin and diadinoxanthin with diatoms and chlorophyll b with green algae [Fiksdahl and Liaaen-Jensen 1988]), but microscopic work conducted suggested that their overall occurrence was uncommon at both Tijuana Estuary and Mission Bay. Occasionally, euglenoids have been seen in high densities at other wetland sites (Underwood 1994). *Vaucheria* patches are known from mudflats just southeast of vegetated salt marsh in northeastern Mission Bay, but these organisms don't appear to be regularly found in other kinds of wetland habitats at this site. Like the Euglenophyceae, *Vaucheria* is known from a number of

intertidal wetland locations (Polderman 1978, Peletier 1996), but its overall distribution or frequency of occurrence is poorly understood.

Diversity, composition, and productivity

Although some recent terrestrial work suggests that reductions in biodiversity at small scales may result in reductions in ecosystem processes such as biomass accumulation or resistance to exotic species invasions (Naeem et al. 2000, Tilman et al. 2001), the role of biodiversity in ecosystem function is less clear in marine habitats. For example, in some shallow water coastal communities, it appears that the presence of particular species can have disproportionate effects on the structure (Paine 1966, Dayton 1975) or energetic processes (Paine 2002) of marine assemblages, implying that in many ecosystems the effects of variable species identity might outweigh effects due to species diversity alone. In such communities dominated by "keystone" (Power et al. 1996) or "foundational" species (Dayton 1972), loss of only a single taxon may be linked to substantial ecological change. As a corollary, the loss of other species might have little impact on functional attributes.

In the microproducer communities studied here, diversity generally did not predict rates of gross productivity and was only positively associated with standing stocks of microproducers in vegetated salt marsh sediments. Communities of lower functional diversity had photosynthetic rates equal to or exceeding those of more diverse communities except for laboratory cyanobacterial cultures which were significantly outperformed by communities composed of three functional groups (Figure 4.10). In terms of gross productivity, these data suggest a high degree of functional redundancy among the major groups of marine wetland microproducers

studied, implying that loss of one or more groups would not substantially alter sediment productivity.

A positive association between functional group diversity and biomass in halophyte salt marsh, suggests some positive influence of diversity on sediment primary production. Anoxygenic photobacterial density and possibly green algal abundance may help drive increases in biomass (Figures 4.6, 4.9). Perhaps community biomass increases as these various organisms fill available niches on the sediment surface (green algae) and at depth where sediments receive sufficient light but are anoxic (anoxygenic photobacteria). Even if wetland production is largely un-impacted by variation in diversity or composition, other processes like nitrogen fixation are likely to depend heavily on which taxa are present in assemblages.

The distribution of bacteriochlorophyll a within the sediments studied suggest an important role for anoxygenic bacteria in wetlands. Virtually all sediments investigated contained at least trace quantities of bacteriochlorophyll a indicating the presence of purple phototrophic bacteria, with possibly the addition of some green phototrophic bacteria (Imhoff 1999). Some sediments contained substantial photobacterial populations. In unvegetated sediments collected from Mission Bay and Tijuana Estuary during spring 2002, for example, mean bacteriochlorophyll a/chlorophyll a ratios were <0.01, but mean ratios rose to 0.05 and 0.08 for Mission Bay mixed halophyte marsh and *Spartina*-dominated marsh respectively. In fact, sediment assemblages collected from upper intertidal halophyte marsh in Mission Bay during summer 2004, had a mean bchl a/chl a ratio of 0.22 (n=33), and two individual assemblages approached ratios of 1.0! (Cores collected for this study were held in the lab, some under anoxic conditions, from days to about 1.5 wk, likely enhancing anoxygenic bacterial populations relative to other phototrophs.)
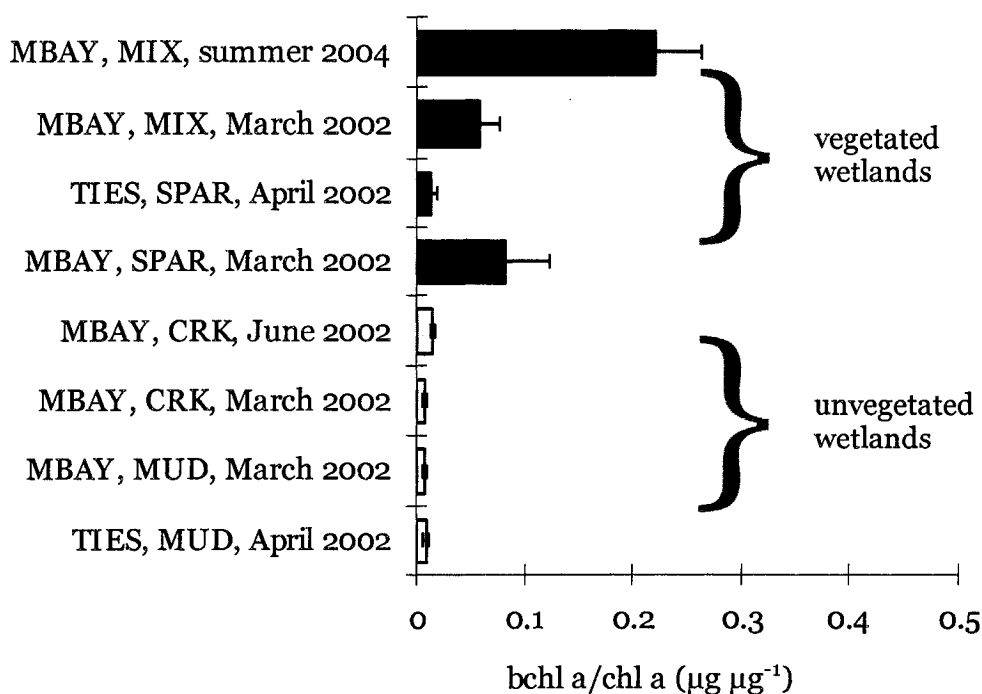
Figure 5.3. Mean (±1 S.E.) bacteriochlorophyll a to chlorophyll a ratios (bchl a/chl a) in sediments from various wetland habitats in Mission Bay (MBAY) and Tijuana Estuary (TIES). Only natural wetland sediments are included. MIX = mixed halophyte salt marsh, SPAR = *Spartina* salt marsh, CRK= unvegetated creek-banks, MUD = mudflat.

Several researchers have postulated that bchl a/chl a ratios can be used as a proxy to estimate the relative contributions of anoxygenic and oxygenic producers to total ecosystem carbon fixation, assuming that carbon fixation rates per bchl a and per chl a concentrations are similar. Wetland sediment bchl a/chl a from the present study lends support for two important conclusions. First, anoxygenic production may be relatively more important in shallow water marine sediments than in many open ocean euphotic zones, where bchl a/chl a may range from about 0.01 to 0.10 (Kolber et al. 2001, Goericke 2002). However, marine wetland rates may be comparable to certain freshwater lakes (Takahashi and Ichimura 1968, Steenbergen et al. 1987)

where anoxygenic/total production falls on the higher end of the approximately 0.3-80% global range for aquatic systems (Canfield et al. 2005). Second, within wetlands, anoxygenic photosynthesis could be a more important portion of total ecosystem carbon fixation in vegetated salt marshes that in unvegetated habitats (Figure 5.1).

Despite these conjectures, it is not yet clear whether sediment concentrations of bacteriochlorophyll a versus chlorophyll a can be used to accurately reflect true differences between anoxygenic versus oxygenic photosynthesis or whether many other marine sediments have bchl a/chl a as high as many of the vegetated sediments observed in this study. For instance, since some groups of anoxygenic photobacteria are capable of growth by assimilation of organic compounds (they are not *obligate* photoautotrophs), the relatively large pool of organic matter in sediments may provide much of the energetic needs of these organisms; anoxygenic photosynthetic rates may thus be low even though bacteriochlorophyll concentrations are high (Kolber et al. 2001).

The importance of composition

I found little apparent role of diversity for microphytobenthic productivity, but several other important wetland functions are mediated by wetland algae and bacteria. Fluxes of nutrients between sediments and the overlying water column (or air, during emersion) may be modified by the kinds of microphototrophs resident. The density of prokaryotic producers would seem to be an obvious case: rates of nitrogen fixation and anoxygenic photosynthesis are expected to decrease as their abundance goes down. Indeed, since research suggests that approximately 73-82% of salt marsh nitrogen requirements are met by diazotrophy (Canfield et al. 2005), the

spatio-temporal variation in the abundance of phototrophic nitrogen fixers is likely to directly impact ecosystem processes. Furthermore, changes in composition may influence the erosion threshold of wetland sediments. Even though virtually all kinds of sediment microproducers exhibit some positive effect on sediment stability (Paterson 1997), Scoffin (1970) found that *Enteromorpha* mats were superior to sediments with cyanobacteria or rhodophytes in terms of sediment cohesion. Likewise, Holland et al. (1974) experimentally documented species-specific differences in sediment cohesion in various benthic diatoms.

Less obvious are the possible complex interactions between different microphytobenthic groups and sediment bacteria and infauna. Several researchers have hinted at the possible presence of active microbial consortia in sediments that might enhance the efficiency of carbon and nitrogen metabolism in sediments (Paerl et al. 1993, Pinckney and Paerl 1997; see also Boetius et al. 2000 for deep-sea microbial communities). If tight linkages between metabolic processes exist in benthic microbes, loss of any functional group may likely lead to a break down of energy and material transport. In addition, sediment invertebrates depend to a large degree on wetland microalgae as a food source and to the extent metazoans specialize on certain microphytobenthic groups, floral composition obviously becomes and important factor in wetland food webs dynamics.

Unfortunately, marine wetland ecologists have often treated microphytobenthic organisms as a single cohesive ecological unit and approached quantification of these communities via measurement of chlorophyll a (e.g., Posey et al. 1995, Lever and Valiela 2005). Chlorophyll a measurements alone provide no insight into the composition or biological diversity present in microproducer assemblages. As demonstrated in this dissertation, standing stocks of

microproducers can be weakly correlated with functional diversity (Figures 4.6, 5.4) or with the relative abundance of cyanobacteria versus diatoms (Figure 5.5), but these relationships are (a) characterized by much unexplained variation and (b) may be heterogeneous in strength and direction under different environmental contexts. This implies that measures of chlorophyll a have little predictive power about the kinds of organisms present in sediments.

Various tools are available to help elucidate the structure of microproducer communities at finer taxonomic resolution. Light and electron microscopy, laboratory culturing, and nucleic acid sequencing can be used for study of community composition and diversity at the smallest taxonomic scales. Unfortunately, some of these techniques may not be feasible for routine study by wetland ecologists (who often need large sample sizes) because of cost (EM) or time (culturing) constraints. Study of microphytobenthos at the functional group level may offer an attractive alternative to ecological studies at fine taxonomic resolution. Characterization of functional group composition and diversity by light microscopy can be done cheaply and without comprehensive taxonomic expertise. Pigment-based approaches, though more costly, also provide valuable information on major sediment functional groups. Pigment analyses are sensitive to low taxon abundances (rare organisms which might be missed by microscope work), are much more precise quantitative measures of community composition, and can also provide physiological information about microphytobenthic assemblages (e.g., the diatoxanthin to diadinoxanthin ratio is a measure of light-induced xanthophyll cycling).
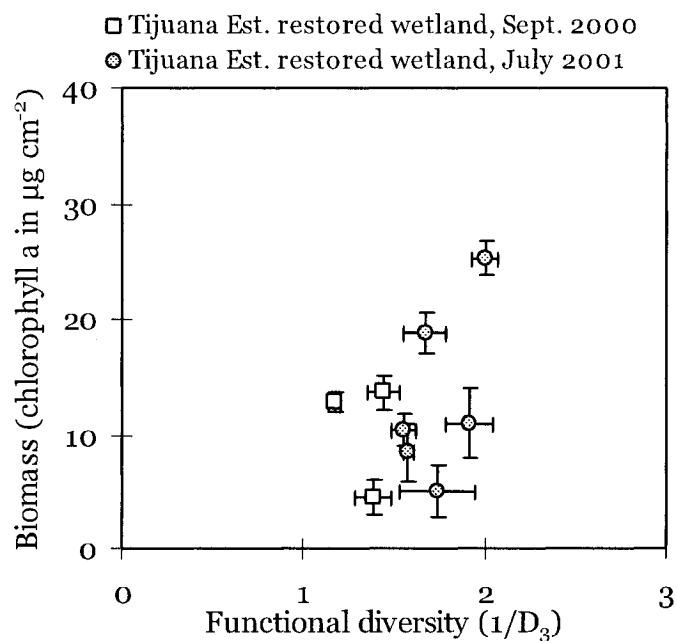
□ Tijuana Est. restored wetland, Sept. 2000
⊘ Tijuana Est. restored wetland, July 2001

Figure 5.4. Microphytobenthic functional diversity versus biomass in marine wetland sediment communities collected from Tijuana Estuary restored wetland patches (see Chapter III). Points represent means (±1 S.E.) of individual communities.

□ Tijuana Est. restored wetland, Sept. 2000
⊘ Tijuana Est. restored wetland, July 2001

Figure 5.5. Cyanobacteria to diatom ratios versus biomass in marine wetland sediment communities collected from Tijuana Estuary restored wetlands patches (see Chapter II). Points represent means (±1 S.E.) of individual communities.
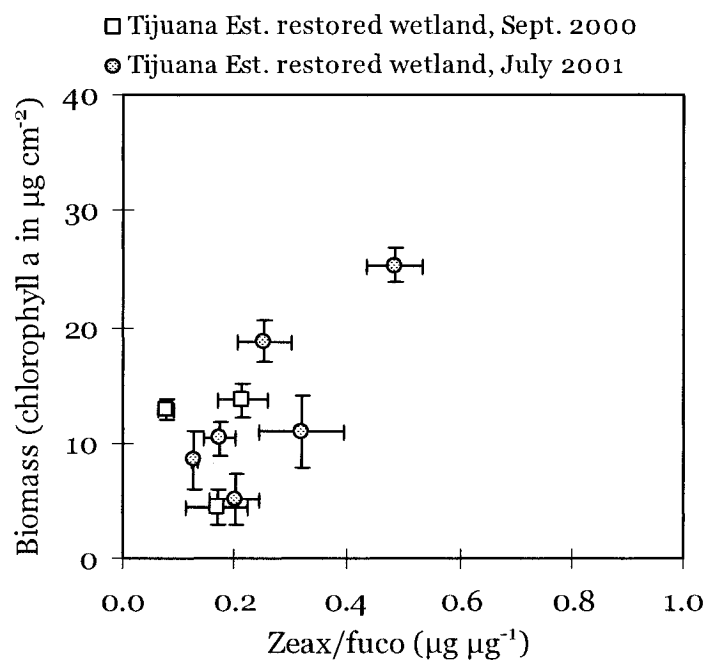
A conceptual model of microproducer composition

Because of what we now know regarding spatial variation in microproducer composition, and the likelihood of functional specialization by different taxonomic groups, I propose a conceptual model that characterizes how community composition may relate to ecosystem-wide processes in southern California wetlands. In this model, tidal sediments are composed of a mosaic of patches of different microproducer composition and diversity. More diverse, cyanobacteria and anoxygenic photobacteria-rich assemblages tend to occur in organic-rich and low light sediments (e.g., salt marsh) or in warm, shallow-water pools. These patches are characterized by higher rates of nitrogen fixation, sulfide oxidation, and anoxygenic photosynthesis (especially during anaerobic conditions) than other sediments. Green algae also occur in highly localized, but relatively uncommon, patches that are sometimes transported around wetland habitat by hydrodynamic forces. These macroalgal rafts are sites of high rates of oxygenic photosynthesis but tend to reduce the biomass of other benthic phototrophs below them (Figures 2.2, Figure 4.10, Sundbäck et al. 1996b). Finally, the most prevalent wetland patches consists of rich diatom communities characterized by moderate rates of biomass-specific production and relatively low nitrogen fixation. Because of their sheer ubiquity across intertidal sedimentary landscapes, these diatom assemblages account for the bulk of total wetland carbon fixation. Seasonal variation in composition (with green algae more prevalent during cool months and cyanobacteria more common in the summer) contributes to changes in the spatial mosaic over time.

Using this model, several important hypotheses emerge. First, nutrient enrichment of wetland waters may lead to blooms of green algae and increases in ecosystem wide oxygenic production. Second, habitat and site-specific concentrations of organic matter in sediments (possibly present as detrital material) may control spatial patterns of cyanobacterial and photobacterial abundance. These hypotheses need to be tested by simultaneous measurement of composition and function various wetland habitats and by controlled experiments.

Future directions

While much progress has undoubtedly been made in the ecology of wetland microproducers over the last several decades, to better understand variation in the processes mediated by microproducer communities, it is important that ecologists gain greater appreciation for the role of composition and diversity in microphytobenthic community ecology. This will involve efforts by wetland ecologists (whether microalgal specialists or not) to measure more than just aggregate features of microphototrophs such as sediment chlorophyll a concentrations. Modest investments of time and resources can help elucidate composition and diversity at least at coarse taxonomic resolution. Pigment-based techniques (HPLC), genetic analyses (rRNA), culturing, and light microscopy are available for investigation of community composition in these cryptic floras.

More work at a variety of taxonomic levels within microproducer assemblages will help sharpen our understanding of wetland ecosystem structure and processes. Additionally, laboratory and experimental tests will be needed to better elucidate the nature of interactions between microproducers and other wetland biota (e.g., see

Risgaard-Petersen et al. 2004) and how the nature of interactions vary according to environmental context (e.g., see Van Der Grinten et al. 2001).

Several additional experiments would shed more light on the results seen here. First, additional manipulative work is needed to understand the biotic and abiotic controls on the composition and diversity of the major functional groups present in wetland sediments. A variety of abiotic factors (salinity, sediment chemistry) and biotic processes (herbivory, bacterial-algal competition) are likely to contribute to variation in community composition, but the work conducted here suggests two variables which may be major players: light and sediment organic matter. Well-replicated shading or enrichment experiments in intertidal mudflats, for example, could help elucidate the separate and combined effects of these factors on community composition and diversity.

Because intertidal wetlands are also known to be stressful transition zones (Levin et al. 2001) with steep abiotic gradients (Figure A2.8; Janousek and C. Whitcraft, unpublished data) it is desirable to determine whether these gradients control the relative abundance of major phototrophic groups and their functions (photosynthesis, nitrogen fixation). Furthermore, gradients in salinity, desiccation or nutrient availability could be used to test for stress-related changes in the relationship between microproducer diversity and function and to investigate the prevalence of positive interactions in microproducer assemblages.

As diversity and composition are integrated into conceptual models of wetland ecosystems, we will be better able to answer these fundamental questions: (1) How do natural and anthropogenic disturbances influence these basal members of wetland food webs?, (2) Does spatial and temporal variation in composition lead to spatial and temporal variation in ecosystem processes? and (3) What maintains the diversity and

resilience of coastal wetland ecosystems? Knowledge of these and other questions will enable ecologists to better address the consequences of various mitigation practices at the ecosystem level and allow resource managers and the public to better protect, restore, and enhance the functions of our valuable coastal marine wetlands.

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Appendix 1.

A taxonomic guide to the benthic algae and cyanobacteria of the

coastal marine wetlands of southern California

Introduction

The coastal marine wetlands of California host a variety of algal and bacterial producers in addition to the conspicuous vascular plant flora. These producers include cyanobacteria, phototrophic bacteria, diatoms, green algae, and other eukaryotic algal groups such as the tribophyte, *Vaucheria*, euglenoids, and red and brown seaweeds (Sullivan and Currin 2000, Barnhardt et al. 1992). Most of the coastal wetland taxa resident in or on sediments are microscopic (or at least diminutive in habit) and are often more or less overlooked in marine algal floras (e.g., Abbott and Hollenberg 1976).

Early research on the taxonomy of California wetland algae began with Setchell, Gardner, and Hollenberg (Gardner 1918, Setchell and Gardner 1919, Purer 1942). Identification, lists and/or descriptions of cyanobacteria and macroalgae from salt marshes and mudflats were compiled in these works, but these floristic accounts principally covered either rocky shore cyanobacteria (Gardner 1918, Setchell and Gardner 1919) or wetland vascular plant floras (Purer 1942). Several decades later, Zedler (1982) published a list of cyanobacteria, diatoms, and green algae collected from Tijuana Estuary at the southern extreme of the state. She recorded 32 species of diatoms, 4 cyanobacterial taxa, and the green algae *Rhizoclonium* and *Enteromorpha*, but did not provide a comprehensive account of her observations at that time. Carpelan (1978) studied pelagic diatoms in four lagoons in northern San Diego Co., but comparison of his list with Zedler (1982) suggests few overlapping species. Other species-level information on the southern California wetland microflora can be found in Wilson (1980). Records of wetland macroalgae have been compiled for Humboldt Bay (Barnhardt et al. 1992), and for Newport Bay in southern California (Vogl 1966).

Stewart's (1991) treatment of San Diego county seaweeds also contains wetland occurrences of marine macroalgae.

Despite these varied efforts, no attempt to compile a comprehensive inventory of benthic microproducers has apparently been made for the southern California region. Each major phylogenetic group present in wetland sediments presents its own unique challenges for ecologists seeking to conduct studies at the species or generic levels. For diatoms, identification to the species level is largely dependent upon frustule (skeletal) ornamentation (Round et al. 1990), information best acquired via electron microscopy. Successful diatom identification by light microscopy is hindered by the poor resolution of frustule decoration, but it can still be useful for determination of plastid morphology, another important characteristic in diatom identification (Round et al. 1990). Additionally, since no recent local reference flora exists for the diatoms, application of nomenclature to local species is dependent upon the work of systematists working in other regions.

Work on a comprehensive inventory of benthic marine cyanobacteria for California wetlands is also fraught with challenges. First, ecologists and systematists interested in the cyanobacterial flora must decide between several competing classification schemes (Sullivan and Currin 2000). In cyanobacterial classification, the most fundamental dichotomy lies between the "botanical" (morphology-based) and "bacterial" (culture-based) approaches (Whitton and Potts 2000). Furthermore, within the botanical approach, there are two major historical trends in classification - one centered around the traditional work of Geitler and its reappraisal by Anagnostidis, Komárek and others (e.g., Anagnostidis and Komárek 1988), and the other representing a radical condensation of several thousand cyanobacterial taxa into a few species by Drouet (Whitton and Potts 2000).

Many modern cyanobacteriologists appear to reject the systematic perspective of Drouet (e.g., Anagnostidis and Komárek 1985), but unfortunately some researchers working in marine habitats several decades ago seemed to favor it (e.g., Humm and Wicks 1980, Zedler 1982), and so in such floristic accounts it becomes difficult to know the original traditional name of the taxa to which they have applied the condensed Drouet system. The bacteriological approach is based on the maintenance of axenic monospecific strains in culture (Whitton and Potts 2000), and is amenable to gathering molecular and physiological data on certain taxa, but only a small fraction of organisms seen in nature are currently in culture (Golubic 1979; Castenholz 2001). Strict adherence to the bacteriological approach would be nearly impossible for working ecologists who are likely to find myriad forms not yet present in cultures. Finally, regardless of systematic approach used, compilation of local cyanobacteria taxa is dependent (like the diatoms) on treatments from geographically distant areas (e.g., Komárek and Anagnostidis 1999), or on accounts consisting primarily of freshwater and terrestrial material (e.g., Desikachary 1959).

Despite these challenges, wetland microalgal systematics presents abundant opportunities for progress in biodiversity studies simply because of the paucity of information available on these producers. In addition, key questions in modern systematics such as the prevalence of cryptic taxa in nature and the degree of congruence between molecular versus morphologically-based phylogenies (Whitton and Potts 2000) could profit from detailed microalgal study. Furthermore, a deeper understanding of the diversity of microproducers present in coastal wetland habitats is essential to a better understanding of ecological interactions between microphytobenthos and other wetland organisms and for the use of biodiversity measures as a means of assessing ecosystem health and dynamics.

In this appendix, I illustrate and describe common benthic microalgae and seaweeds found in or associated with sediments of southern California wetlands (mudflats and salt marshes). Collections were made in Tijuana Estuary and Mission Bay, San Diego County but species of algae previously recorded from wetlands throughout the southern California region (Pt. Conception, Santa Barbara Co. south to Tijuana Estuary, San Diego Co.) are also included. The goal is to provide preliminary, not comprehensive, documentation of the local flora and thus create a stepping stone towards more exhaustive floristic work. Organisms included here were assigned tentative genus (and sometimes species) names based on morphological features visible by eye or by light microscopy. Supporting references pertinent to the identification of taxa, their local distribution, and (where possible) their natural history observations are also included. Documentation of the cyanobacterial flora is more thorough than for other groups.

Materials and methods

Collections of benthic sediment and macroscopic algae were made from 1999-2005 at the Kendall-Frost Mission Bay Marsh Reserve and adjacent Northern Wildlife Preserve in northern Mission Bay, San Diego County (32°47' N, 117°13' W) and from 2000-2001 in Tijuana Estuary (32°34' N, 117°7' W). Various intertidal wetlands are present at these locations including unvegetated mudflat and primary and higher-order creeks, salt marsh vegetated by *Spartina foliosa, Monanthochloe littoralis* and *Distichlis spicata* (Poaceae), *Salicornia bigelovii* and *S. virginica* (Chenopodiaceae), *Batis maritima* (Bataceae), *Triglochin* sp(p)., *Limonium californicum, Jaumea carnosa* (Asteraceae), and *Frankenia grandifolia*

(Frankeniaceae), and low intertidal seagrass beds. Prior to significant coastal development in the San Diego region, Mission Bay supported much more extensive areas of salt marsh habitat, including coverage of the entire southern portion of the Bay and portions of the western and northwestern part of the Bay that was lost during the mid-1900s (USGS 1930, 1967). Although historical maps suggest that the Kendall-Frost Reserve and immediately adjacent areas have long been vegetated salt marsh, this patch of wetland habitat too has decreased in size since the 1930s. Tijuana Estuary has been less impacted by human encroachment.

Photographic documentation and observations were made on live organisms or (occasionally) on organisms maintained in culture at Scripps Institution of Oceanography. Organisms maintained on field sediment and in culture were kept in the laboratory under incubation (usually 21.0-22.0°C; 13 hr light cycle; ~70 µE m^{-2} s^{-1} illumination). Cultures were grown on sterilized f/2 media (ccmp.bigelow.org) prepared in artificial seawater with or without sterilized glass particles (13-44 µm diameter) as substrate. Organisms from field-collected sediment were kept and observed up to ~7 mo following removal from the field (living taxa were still apparently abundant although proportional abundances and composition of taxa may have changed over time). Photographs were taken with a digital camera through compound microscopes (usually at 160 or 400x).

Diatoms were identified to genus where possible using Round et al. (1990). Cyanobacterial taxa were generally identified to genus-level (occasionally species level) using the recent taxonomic treatments in Boone and Castenholz (2001) and Anagnostidis and Komárek (1988, 1989). Humm and Wicks (1979), Desikachary (1959), and Setchell and Gardner (1919) were also consulted for identification and nomenclatural purposes.

Macroalgae attached to sediment-associated substrates or occurring loosely in wetland habitats were pressed on herbarium paper (without prior formalin fixation) and dried. Identification of material was made using Abbott and Hollenberg (1976) and Gabrielson et al. (2004).

Results

EULENOPHYTA

Euglena or *Eutreptia*

Euglenoids have been occasionally observed from wetland sediments in Tijuana Estuary and Mission Bay. Underwood (1994) noted the presence of *Euglena deses* in sediments from the UK, a species that lacks a flagellum. One of the specimens below appears to have at least one flagellum but flagellar shape and number are unknown.
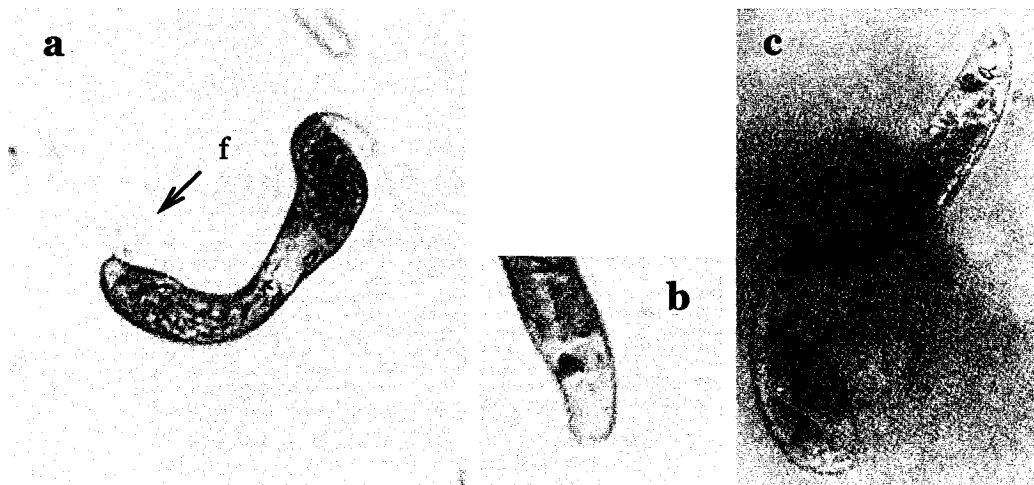
Figure A1.1. An unidentified euglenoid collected from a cyanobacterial mat in Mission Bay sediments during October 2003. (a) Whole organism; f=flagellum. (b) Reddish eyespot of organism in (a). (c) Euglenoid collected from vegetated salt marsh in Mission Bay during summer 2004.

CYANOBACTERIA

Cyanobacteria are frequently found in a variety of coastal marine habitats (mudflats, salt marshes, hypersaline waters, rocky intertidal) throughout the world (Fogg et al. 1973). They comprise an important component of the microscopic flora of southern California salt marshes and mudflats (Zedler 1982), but are often patchy in their spatial distribution (Chapter II).

Previous published records from salt marshes, mudflats, and other coastal wetlands in southern California (Purer 1942, Zedler 1982) have documented the presence of at least 5 taxa distributed in the Oscillatoriales, one of five major groups currently recognized in cyanobacterial taxonomy (Table A1.1). Setchell and Gardner (1919) list many additional taxa from throughout the coast of the western Pacific, but it is uncertain which of their taxa were present in wetland habitats from southern California. Zedler (1982) listed 3 "*Schizothrix*" species from Tijuana Estuary (apparently using the Drouet system), but without descriptions or illustrations, reassignment is not possible. Here, at least 15 "morphospecies" are included from collections in Mission Bay and Tijuana Estuary, San Diego County.

NOSTOCALES

Heterocyst-bearing cyanobacteria are placed in the Nostocales in both traditional and more recent systematic work (=*subsection IV* of Boone and Castenholz 2001). Filamentous, unbranched forms have been collected from both Tijuana Estuary and Mission Bay but they have never been observed in great abundance in sediment material. Several different species may be present in the southern California flora: several *Nodularia*-like taxa are illustrated in Figure A1.2 and a single collection of *Anabaena* (Mission Bay) is depicted in Figure A1.3. Notably, heteropolar Rivulariaceae like *Calothrix* appear to be absent or very rare at these wetland sites, though they are known from a number of intertidal wetlands including *Spartina* shoots (Currin and Paerl 1998) and sandflats (Stal et al. 1985). Interestingly, no records of Nostocales in southern California salt marshes or mudflats appear to have been published to date.

Anabaena sp 1. (?)

The most common Nostocales observed in southern California wetlands are filaments composed of roughly spherical to compressed (discoid) cells with heterocysts not much larger in diameter than vegetative cells. Despite the presence of compressed vegetative cells in these specimens, which would align them with *Nodularia* (Komárek and K. Anagnostidis 1989), heterocysts are spherical, suggesting placement in *Anabaena*. These specimens may correspond better with *Nostoc*, but since akinetes have not been observed in any field material, it is nearly impossible to decide between the two genera.
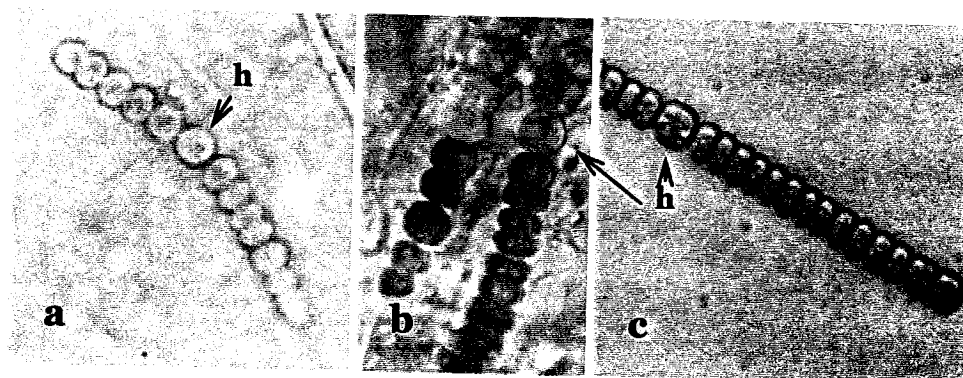
Figure A1.2. Heterocyst (h) bearing cyanobacteria from Mission Bay wetlands tentatively placed in *Anabaena*. (a) Short filament bearing a single heterocyst collected from Mission Bay in October 2003. (b) Filaments epiphytic on *Ulva* (Chlorophyta). (c)

Anabaena sp. 2 (?)

The specimen illustrated here is distinct from other collections because of the presence of enlarged heterocysts. Like the specimens above (Figure A1.2), it may also be more accurately placed in *Nostoc*.
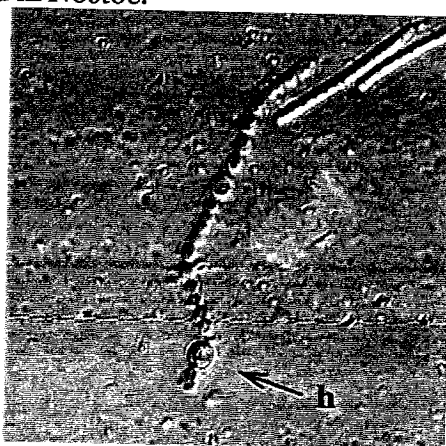

Figure A1.3. Single collection of this species made in Mission Bay from a pool near salt marsh during April 2002. No scale available; h=heterocyst

OSCILLATORIALES

The Oscillatoriales (*subsection III* of Boone and Castenholz 2001) are frequently the most species rich cyanobactarial order in marine wetland habitats. Members are characterized by unbranched, cylindrical trichomes lacking heterocysts. Oscillatoriales may reproduce via short filaments termed hormogonia.

Spirulina spp.

Three genera of regularly twisted cyanobacteria have been reported from marine environments: *Spirulina*, *Arthrospira* and *Halospirulina*. *Spirulina* is distinguished from the somewhat morphologically similar *Arthrospira* by smaller cell diameters, a more compacted coil (as opposed to the loose coil of *Arthrospira*), and cell walls barely visible under light microscopy (Castenholz et al. 2001). However, older taxonomic work (e.g., Umezaki 1961) and even more recent cyanobacterial floristics (e.g., Silva and Pienaar 2000) place both loose and tight-coiled cyanobacteria in *Spirulina*. Members of this genus may have coil diameters up to 12 µm (Boone and Castenholz 2001). Individual filaments show movement either by twisting about the long axis of the filament or by lateral waving of the end of the filament from side to side (in Boone and Castenholz 2001).

Traditionally, at least two species of *Spirulina* have been recognized in marine habitats, these delineated by cellular and spiral diameters: *S. subsalsa* and *S. labyrinthiformis* (Desikachary 1959, Umezaki 1961). The organisms in Figures A1.4a,b correspond closely with the morphological description of *S. subsalsa*, whereas Figure A1.4c can tentatively be assigned to *S. labyrinthiformis*.
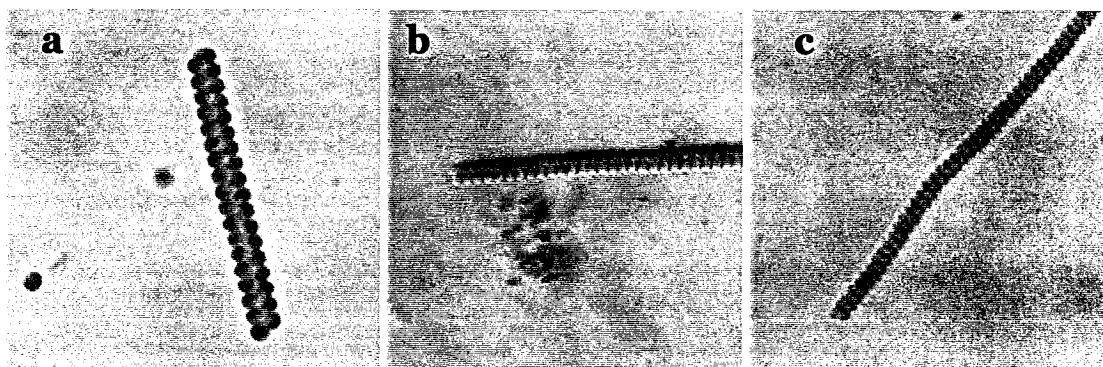

Figure A1.4. *Spirulina* spp. from Mission Bay wetland sediments, October 2003. (a and b) *S. subsalsa*. (a) Coil diameter ~4 µm, (b) Coil diameter: ~4.75 µm, (c) *S. labyrinthiformis*. Coil diameter ~2.4 µm.

Microcoleus

Microcoleus (usually *M. chthonoplastes*) occurs in the sediments of salt marshes and other marine habitats worldwide (Karsten and Garcia-Pichel 1996, Nübel et al. 1999). The taxon illustrated here is also a very common member of both Mission Bay and Tijuana Estuary sediments. Multiple filaments often occupy a single sheath. Cell lengths are slightly longer than widths. Apical cells may be either rounded (Figure A1.5b, c) or conical (not shown).
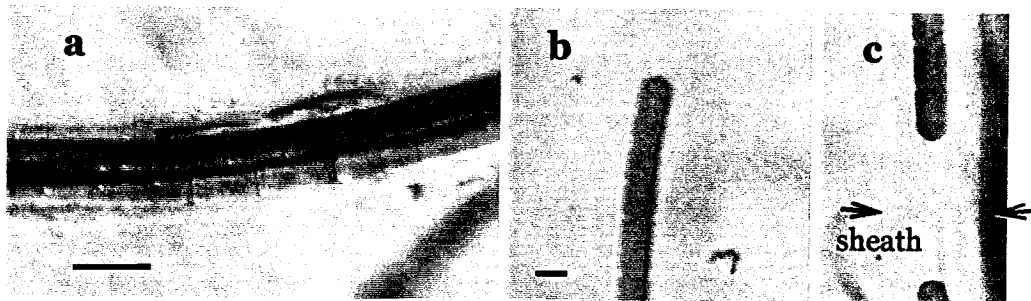

Figure A1.5. *Microcoleus* sp. (a) Bundle of filaments from Mission Bay salt marsh habitat, collected during April 2004; bar= approximately 8.33 μm. (b) Single filament from a cyanobacterial mat in Mission Bay, April 2004; bar=4 μm. (c) Single filament in a larger sheath (faintly visible), collected in October 2003 from salt marsh.

Oscillatoria sp.1

Filamentous, unbranched cyanobacteria with cell length to width ratios <1 are assigned to either *Lyngbya* or *Oscillatoria* (Boone and Castenholz 2001). Both genera may be present in the Mission Bay wetland flora, with up to several species involved. *Lyngbya* can be distinguished from *Oscillatoria* by a relatively thicker sheath (Castenholz et al. 2001a).

The morphological diversity of *Oscillatoria*-like cyanobacteria from Mission Bay wetlands is significant. Setchell and Gardner (1919) record approximately 10 species from wetland habitats on the Pacific coast, though it is unknown how many of their taxa were also resident in southern California.

In the tentative *Oscillatoria* sp. 1, filaments are brownish and are approximately 5-6 µm in diameter. Terminal cells rounded. Constrictions between individual cells are fairly distinctly in this taxon, although it should be noted that cellular constrictions are largely absent in recent conceptions of the genus.
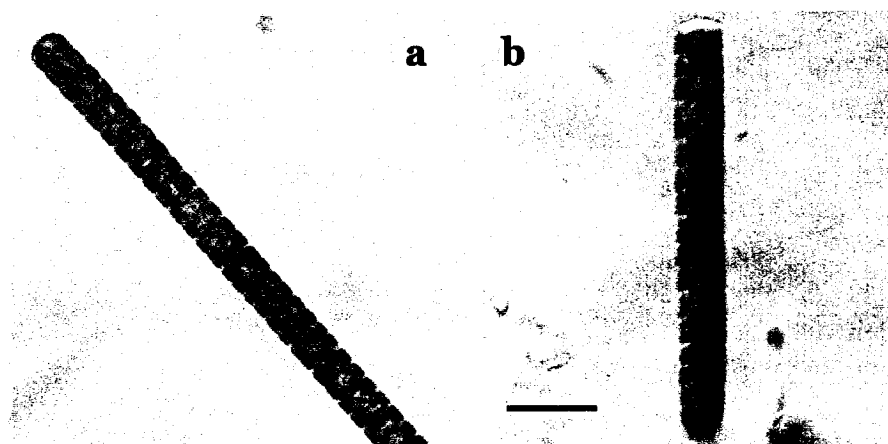

Figure A1.6. *Oscillatoria* sp. 1 (a) Collected in lightly vegetated salt marsh at Mission Bay, October 2003. Filament width ~6 µm. (b) Specimen from a cyanobacterial mat in lightly vegetated salt marsh. Trichome width ~5 µm; bar=10 µm.

Oscillatoria sp.2

Cells in *Oscillatoria* sp. 2 are distinctly granular and are several times wider than they are long. A transparent sheath can be seen protruding beyond the rounded terminal cell (Figure A1.6).
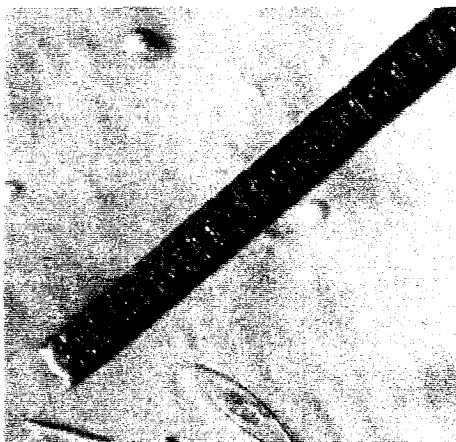

Figure A1.7. *Oscillatoria* sp. 2. Filament width ~9 µm; cells are about 2.5 µm in length.

Oscillatoria sp.3

This is the widest cyanobacterium seen in wetland sediments that roughly corresponds with the generic description of *Oscillatoria*.
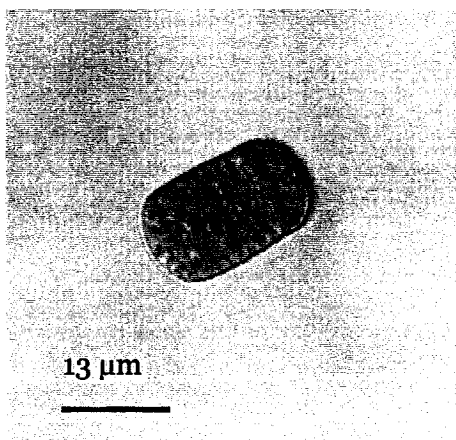

Figure A1.8. *Oscillatoria* sp. 3. Cellular cross walls are faintly visible. Specimen collected during April 2004. Filament width is ~13 µm.

Phormidioideae, sp. 1

Cells is this taxon are approximately isodiametric with shallow inter-cell constrictions. A relatively inconspicuous sheath also appears to be present. Generic placement is difficult because of the lack of observations, however the presence of a sheath appears to preclude placement in *Geitlerinema* (Anagnostidis 1989).
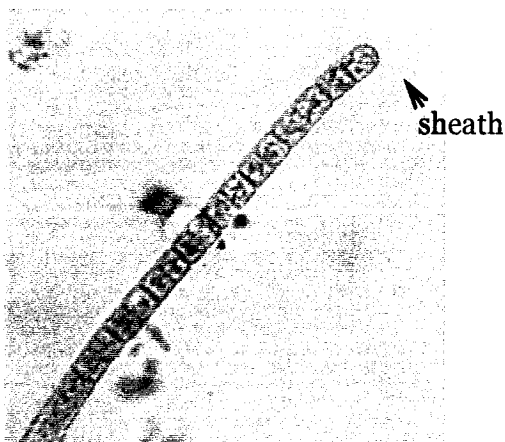

Figure A1.9. Unidentified filament from Mission Bay wetlands (width of trichome ~4.75 μm), of probable affinity to the subfamily Phormidioideae.

Leptolyngbya (?)

Thin (<3 μm wide) filamentous cyanobacteria are common in wetland sediments collected from Mission Bay and Tijuana Estuary. Several species may be present in the local flora but because of their small size, discernment of taxonomically-useful characters is difficult via light microscopy. In one specimen below (Figure A1.11a), cell length to width ratios are approximately two. The specimen is tentatively assigned to the 'provisional' genus, *Leptolyngbya* (Castenholz et al. 2001b), but may likely need reassignment upon more careful scrutiny.

Similar or identical cyanobacteria of ~2.5 μm diameter (Figure A1.11b) have been brought into culture on f/2 media and grow prolifically under incubation (see Chapter IV culture experiment). Cultured organisms are motile.
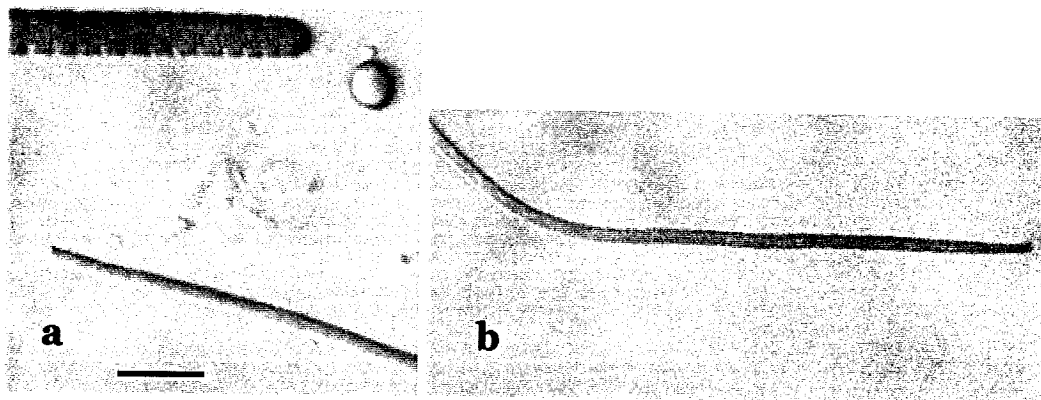

Figure A1.10. *Leptolyngbya* (?) (a) Specimen from Mission Bay sediments, October 2003; bar=10 μm. (b) Specimen from culture.

ORDER PLEUROCAPSALES

The Pleurocapsales (=*subsection II* of Boone and Castenholz 2001) are coccoid cyanobacteria reproducing via multiple fission (Rippka et al. 2001b).

Chroococcidiopsis (?)

Sediment material incubated for a short time in the laboratory following collection from the field yielded abundant coccoid cyanobacteria growing on glass. Based on apparent patterns of cellular division, these organisms have been tentatively placed in the genus *Chroococcidiopsis*.

One or more greenish-yellow cells are present per cluster within a common sheath. There is a large variation in cellular size (from ~1.7 to >24 μm).
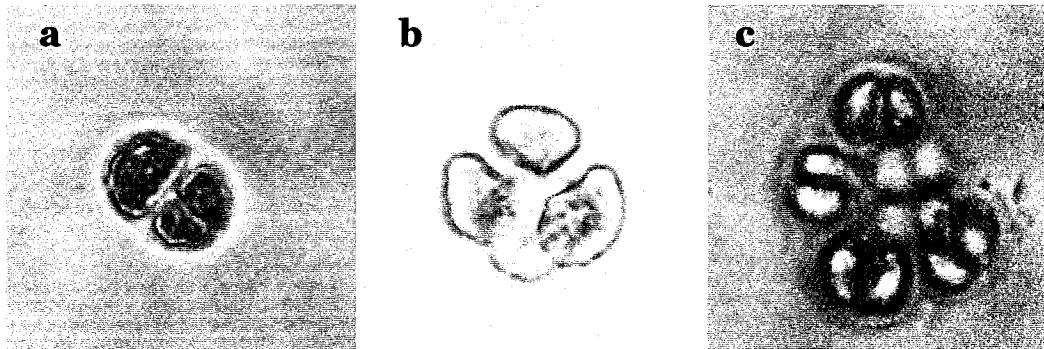

Figure A1.11. Cells tentatively assigned to *Chroococcidiopsis*. (a) Cluster length ~14.5 μm. (b) Tetrahedral-like cell division in 4 cell cluster of ~16 μm diameter. (c) length of cell cluster ~22 μm.

ORDER CHROOCOCCALES

Coccoid cyanobacteria possessing only binary fission as a means of cellular division are placed in the Chroococcales (=*subsection I* of Boone and Castenholz 2001).

Chamaesiphon (?)

As recently defined, *Chamaesiphon* is a unicellular cyanobacterium generally found in attached to substrates in freshwater habitats (Komárek and Anagnostidis 1999, Herdman et al. 2001a). Daughter cells are produced from the larger mother cells by a budding-type binary fission.

The organisms depicted here (Figure A1.12) here have been observed in raw sediment samples and have also been brought into culture (on f/2 media). Cultured cells seemingly do not form clusters of more than about 2 cells. The presence of organisms of similar morphology in coastal wetland sediments suggests that either *Chamaesiphon* is also found from saline habitats or that these organisms represent a morphologically similar taxon to *Chamaesiphon* in need of a new genus.
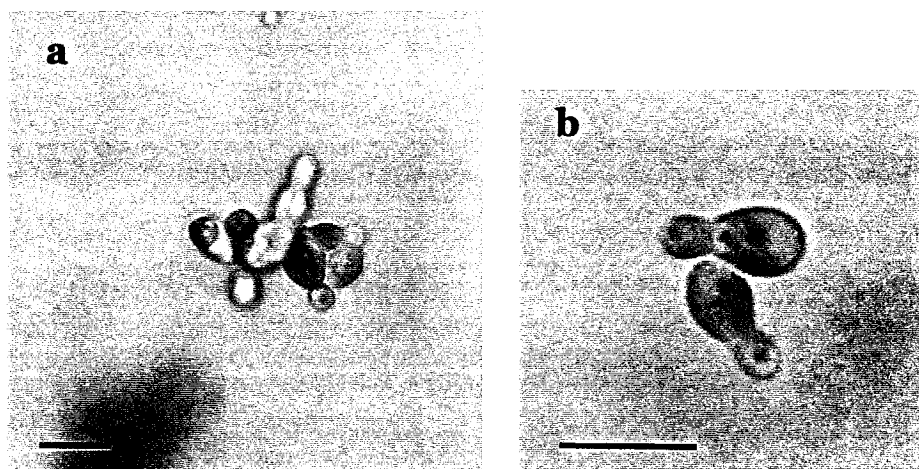

Figure A1.12. Mission Bay sediment phototrophs resembling *Chamaesiphon*. (a) Large cluster of cells. Oblong central cell ~8.33 μm length; bar=8.3 μm. (b) Clusters of two cells; bar=10 μm. Both figures are of sediment-associated material collected during October 2003 and kept in the lab under incubation for about 6 mo.

Chroococcus

    Chroococcus is a freshwater and marine genus of cyanobacteria with large hemispherical cells in clusters of 1-4 (Rippka et al. 2001a). It is similar in general morphology to *Gloeocapsa*, but differs by the possession of larger cell diameters, a more tightly appressed sheath and more hemi-spherical cells (Herdman et al. 2001b). Separation of the two genera by size is not followed by all workers however, (e.g., Silva and Pienaar 2000), even though molecular evidence supports the morphologically-based distinction between these two genera (Rippka et al. 2001a).

    Chroococcus and *Gloeocapsa* are frequently reported from salt marsh habitats worldwide. The large type species, *C. turgidus*, has been previously reported from rocky habitat in California (Setchell and Gardner 1919), but the organism depicted here (Figure A1.13a) may be the first record from salt marsh sediments in California. Other cyanobacteria adhering to the general characteristics of *Chroococcus/ Gloeocapsa* observed in Mission Bay sediments are typically present as clusters of two cells, but four cell organisms have also been observed (Figure A1.14). Organisms with cell diameters >20 µm are herein assigned to *Chroococcus* while those < about 10 µm diameter are placed in *Gloeocapsa*, in accordance with recent bacteriological taxonomic schemes (Herdman et al. 2001b). The ability to fix nitrogen is known from two of the three cultured isolates of *Chroococcus* Rippka et al. 2001a).
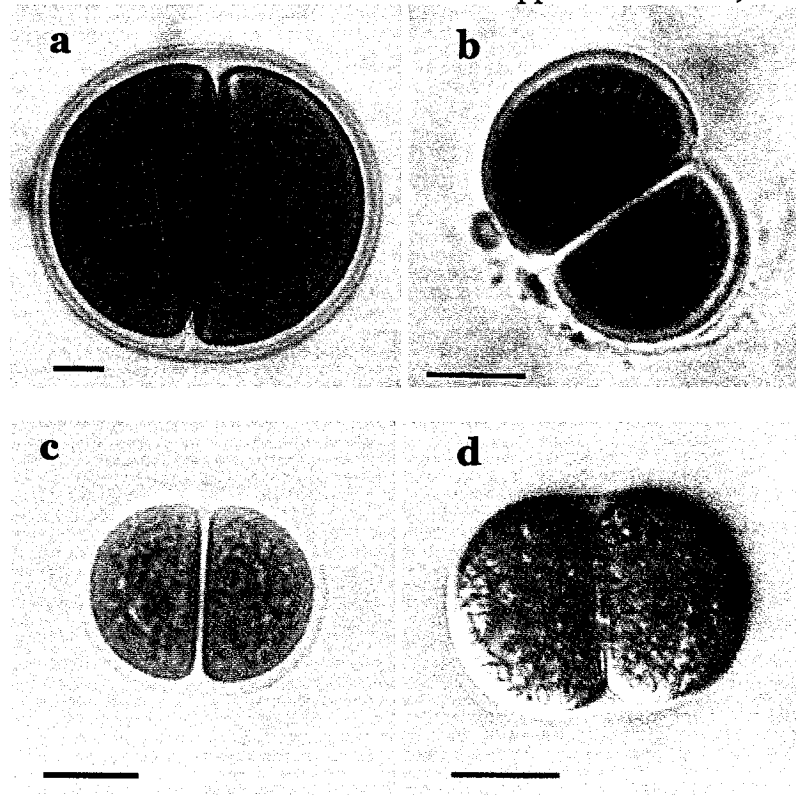

Figure A1.13. Salt marsh *Chroococcus* spp. from Mission Bay. (a) *C. turgidus* from sediments collected during August 2004. (b) *Chroococcus* sp., October 2003. (c) small *Chroococcus*, summer 2004. (d) *Chroococcus* with tightly appressed sheath, collected October 2003. All bars=10 µm.

Gloeocapsa

The two, three, and four celled clusters of cyanobacteria depicted here (Figure A1.14) have been tentatively assigned to *Gloeocapsa*, based on cellular diameters and wider, loosely appressed sheaths (Herdman et al. 2001b). Komárek and Anagnostidis (1999) recognize >30 species in this genus from Europe alone. Nitrogen fixation has been observed in an unknown member of this genus (Wyatt and Silvey 1969).
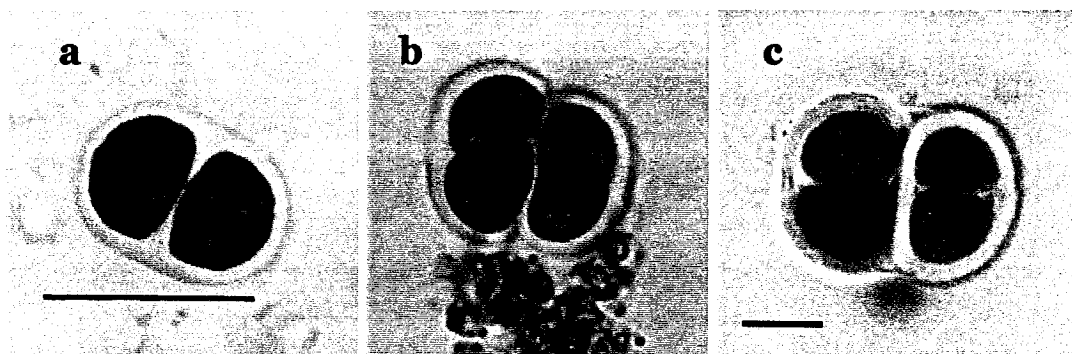

Figure A1.14. Examples of two (a) and four-celled (b, c) colonies of coccoid cyanobacteria tentatively assigned to the genus *Gloeocapsa*. (a) Organism from sediments in lightly vegetated salt marsh. Sheath thickness ~2.5 μm; bar=24 μm. (b) Colony length ~33 μm. (c) *Gloeocapsa* collected from halophyte marsh during summer 2004. Bar=10 μm

Synechocystis (?)

Loose clusters of coccoid cyanobacteria have been occasionally observed in wetland sediments. The organisms below are very tentatively assigned to *Synechocystis* based on their cellular diameter, apparent cell division in 3 dimensions, and absence of a well-structured sheath or gas-filled vesicles (Herdman et al. 2001c).

Figure A1.15. *Synechocystis* (?). A cluster of coccoid to egg-shaped cyanobacteria associated with sediment particles. Cellular diameter is approximately 5 μm; bar=10 μm.

Johannesbaptistia pellucida

This (apparently) rare taxon has only been observed once in Mission Bay wetland sediments. It was collected from vegetated salt marsh habitat during summer 2004. The organism depicted below is similar in morphology to *Johannesbaptistia* as illustrated in Humm and Wicks (1980) and Potts (1980). Cells are arranged in a pseudo-filament (Humm and Wicks 1980). The species been found worldwide from lower latitudes, but is apparently not found in abundance (Umezaki 1961). Sage and Sullivan (1978) provide one additional record of this taxon for North American salt marsh habitats.

Figure A1.16. Portion of *Johannesbaptistia pellucida* filament collected from salt marsh sediments in Mission Bay. The sheath is approximately 2.5 μm thick; bar=10 μm.

Table A1.1. Benthic cyanobacteria in southern California wetlands. Taxonomic structure is after Boone and Castenholz (2001). * tentative identifications. Zedler's (1982) three *Schizothrix* species have not been included; these may well be accomodated by other taxa listed here.

Taxon	Regional occurance	References
Nostocales		
Anabaena sp. 1 *	Mission Bay	this study (Figure A1.2)
Anabaena sp. 2 *	Mission Bay	this study (Figure A1.3)
Oscillatoriales		
Spirulina subsalsa	Mission Bay	this study (Figure A1.4a,b)
Spirulina labyrinthiformis	Mission Bay	this study (Figure A1.4c)
Microcoleus lyngbyaceus	Tijuana Estuary	Zedler 1982
Microcoloeus sp.	Mission Bay	this study (Figure A1.5)
Oscillatoria sp. 1	Mission Bay	this study (Figure A1.6)
Oscillatoria sp. 2	Mission Bay	this study (Figure A1.7)
Oscillatoria sp. 3	Mission Bay	this study (Figure A1.8)
Lyngbya aestuarii f. ferruginea		Purer 1942
Phormidioideae, unknown species	Mission Bay	this study (Figure A1.9)
Leptolyngbya *	Mission Bay	this study (Figure A1.10)
Pleurocapsales		
Chroococcidiopsis *	Mission Bay	this study (Figure A1.11)
Chroococcales		
Chamaesiphon *	Mission Bay	this study (Figure A1.12)
Chroococcus turgidus	Mission Bay	this study (Figure A1.13a)
Chroococcus sp(p).	Mission Bay	this study (Figure A1.13b-d)
Gloeocapsa sp(p).	Mission Bay	this study (Figure A1.14)
Synechocystis *	Mission Bay	this study (Figure A1.15)
Johannesbaptistia pellucida	Mission Bay	this study (Figure A1.16)

CHLOROPHYTA

Green macroalgae are relatively common in southern California tidal wetlands. Many belong to the genus *Ulva* but additional taxa such as *Rhizoclonium* are found (Table A1.2). California Chlorophyta are relatively well represented in wetlands compared with the state's rocky the shorelines where rhodophytes and phaeophytes tend to dominate. Nübel et al. (1999) found the green microalga *Dunaliella* in hypersaline sediments of Ojo de Liebra Lagoon (Baja California), but this taxon has not yet been verified for San Diego County wetlands.

Rhizoclonium spp.

Gabrielson et al. (2004) recognize two *Rhizoclonium* species from the California and Oregon flora: *R. riparium* (Roth) Harvey, with roughly isodiametric cells with widths usually not exceeding 30 μm and *R. tortuosum* (Dillwyn) Kützing with cells several times longer than wide and filament widths of approximately 35 to 40 μm.

Stewart (1991) and Zedler (1982) record *R. riparium* from Tijuana Estuary. The specimens represented here are the first published record of *Rhizoclonium* from Mission Bay. A variety of cellular morphologies are evident: Mission Bay material shows variation in with cell shape, the thickness of cell walls, and cell length to width ratios, which renders species-level assignment based on morphology alone difficult.
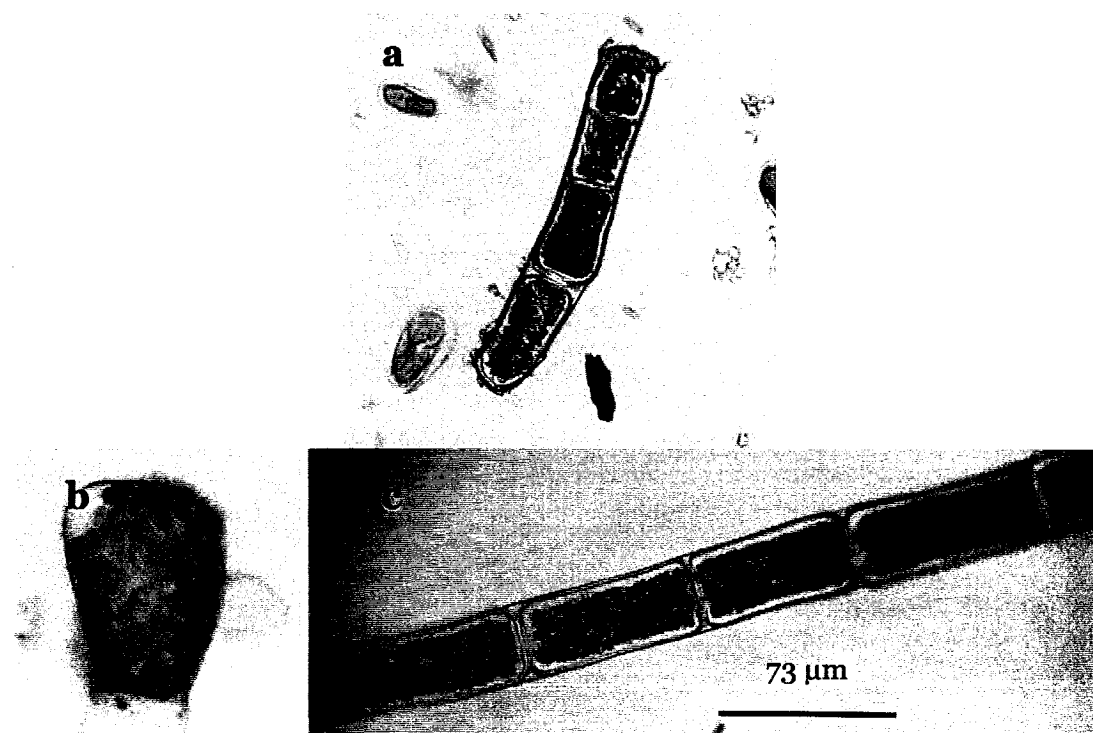

Figure A1.17. *Rhizoclonium* spp. from Mission Bay. (a, b) Short filaments roughly corresponding to *R. riparium*. (c) *R. tortuosum* from salt marsh sediments collected February 2005.

Ulva

A number of *Ulva* and *Enteromorpha* species have been recorded from wetlands in southern California. *Enteromorpha* species were recently transferred to *Ulva* because neither genus is monophyletic using molecular characters (Hayden et al. 2003). I have observed both sheet-like and tubular thalli in Mission Bay wetlands and the following species have been identified in this work: *U. prolifera*, *U. flexuosa* amd *U. clathrata*. Additional records from the literature are given in Table A1.2.
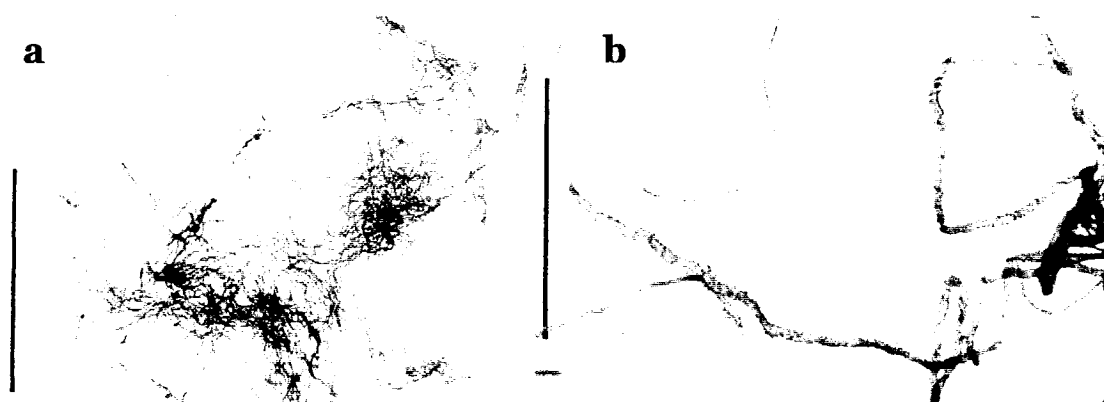

Figure A1.18. *Ulva* spp. (a) Thalli of *U. flexuosa* collected from restored wetland in Tijuana Estuary, February 2000; bar=5 cm. (b) Thalli of *U. clathrata* from Mission Bay during November 1999.

Bryopsis hypnoides Lamouroux

A single specimen of *Bryopsis* was collected from a tidal channel in Mission Bay salt marsh; it is tentatively assigned to *B. hypnoides*. Stewart (1991) makes no mention of the presence of this species in San Diego wetlands.

Figure A1.19. Dried herbarium specimen of *B. hypnoides* collected from Mission Bay during November 1999; bar=5 cm.

PHAEOPHYTA

Macroscopic brown algae are generally rare in temperate marine wetlands. In southern California, only unspecified members of the Ectocarpales have been recorded from soft sediment habitats along the coast (Stewart 1991). Giant kelp wrack (*Macrocystis*) is frequently found in mudflats and marshes, but it is not a component of the living flora. *Fucus* sometimes occurs in the upper intertidal of estuaries in other regions (Gabrielson et al. 2004).

Sargassum muticum (Yendo) Fensholt

A single collection of *Sargassum* was made in an intertidal channel in Mission Bay salt marsh habitat. *S. muticum* is an invasive species from Japan that has spread throughout the west coast of the United States (Abbott and Hollenberg 1976). It is very common on open coast habitats but has also apparently penetrated Mission Bay. Lack of hard substrates at lower intertidal elevations in the local wetlands probably greatly impedes its proliferation there.

Figure A1.20. Portion of *Sargassum muticum* frond from Mission Bay salt marsh creek, November 1999; bar=5 cm.

TRIBOPHYTA

The benthic tribophyte, *Vaucheria* is known from marine wetlands worldwide (see Chapter IV). It is a single celled (coenocitic) dark green alga occurring in moss-like patches on the surface of sediments. The genus has been found in Tijuana Estuary, southern San Diego Bay, Mission Bay, and in the San Diego River channel (Stewart 1991). *V. longicaulis* has been reported from Elkhorn Slough (Monterey Co.; Abbott and Hollenberg 1976), but it is unclear if the local taxon (or taxa) can be assigned to this species. In Mission Bay, *Vaucheria* appears to favor mudflat habitat, especially near the edge of salt marsh vegetation.

RHODOPHYTA

Red algae are very diverse in rocky habitats of temperate and sub-tropical marine coastlines, but are more poorly represented in temperate wetland habitats. A few species occur in mudflat/seagrass habitat in Mission Bay and San Diego Bay (Stewart 1991), but none has apparently been recorded from the local salt marshes. Stewart (1991) lists *Aglaothamnium cordatum* from mudflats in southern San Diego Bay and *Gracilaria* spp. from San Diego County bays. *Dasya sinicola* var. *californica* (Gardner) Dawson has been noted from Newport Bay (Abbott and Hollenberg 1976) and from San Diego Bay (Stewart 1991)
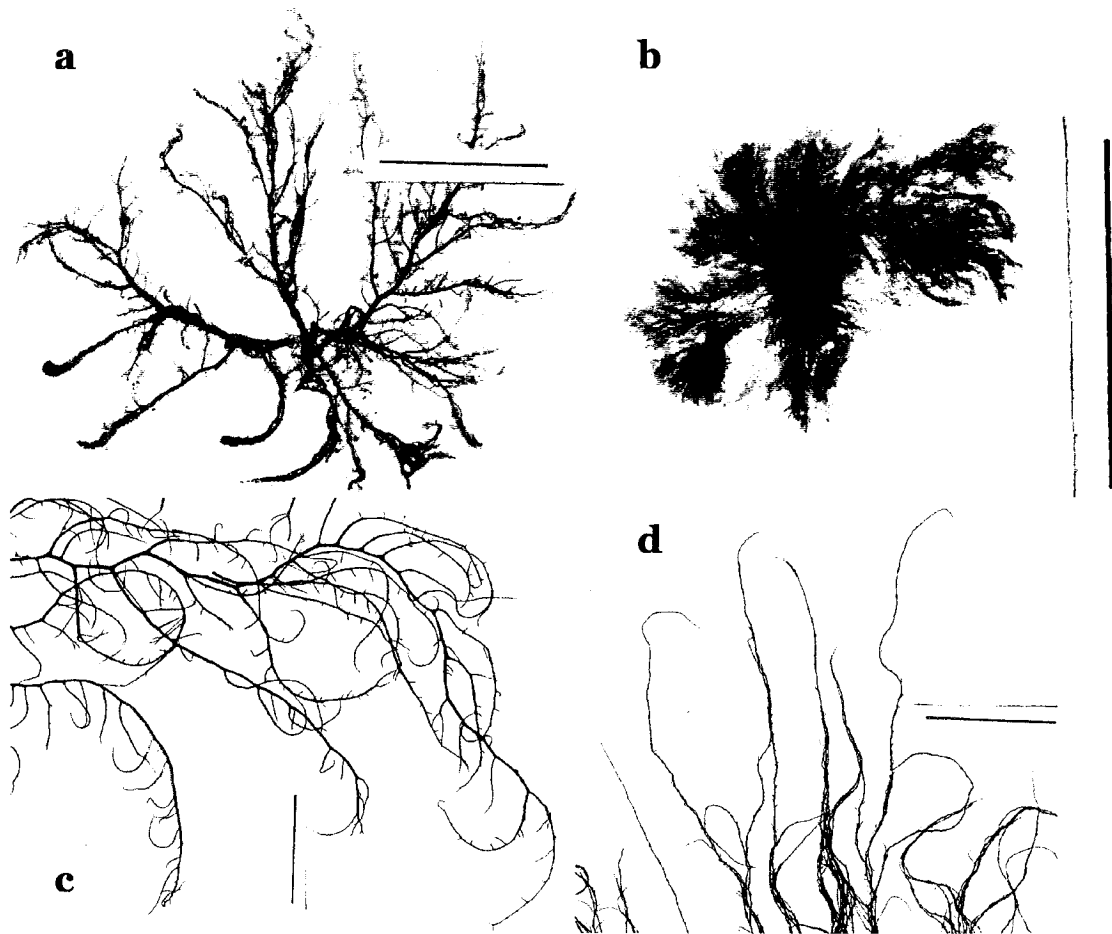

Figure A1.21. Rhodophytes collected from Mission Bay wetlands (non-salt marsh) during November 1999. (a) ? *Dasya sinicola* var. *californica*. (b) Thallus potentially belonging to *Aglaothamnion, Callithamnion* or *Pleonosporium*; bar=5 cm. (c and d) *Gracilaria pacifica* Abbott-like thalli (*G. verrucosa* of Abbott and Hollenberg [1976]). All bars=5 cm.

Table A1.2. Benthic macroalgae of southern California wetlands. The Tribophyta are also known in older literature as the Xanthophyta (e.g., Stewart 1991). * tentative identifications. Attribution of Purer's (1942) "*Enteromorpha*" species to contemporary binomials has not been attempted, although all should now be in the genus *Ulva*.

Taxon	Regional occurrence	References
Phaeophyta (brown seaweeds)		
Ectocarpaceae	San Diego River	Stewart 1991
Sargassum muticum	Mission Bay	this study
Rhodophyta (red seaweeds)		
A. cordatum	San Diego Bay	Stewart 1991
Gracilaria pacifica *	Mission Bay	this study
Dasya sinicola var. *californica* *	Mission Bay	this study
Chlorophyta (green seaweeds)		
Bryopsis hypnoides	Mission Bay	this study
Chaetomorpha spiralis	Mission Bay	Stewart 1991
Cladophora sp(p).	San Diego Co.	Purer 1942, Vogl 1966
Derbesia marina	San Diego River	Stewart 1991
Rhizoclonium riparium *	Mission Bay	this study
R. tortuosum	San Diego Co.	Purer 1942
U. clathrata	Tijuana Est.	this study
Enteromorpha crinita	San Diego Co.	Purer 1942
E. expansa	Tijuana Estuary	Stewart 1991
U. flexuosa	Tijuana Estuary	Purer 1942; this study
U. intestinalis	San Diego Co.	Purer 1942
E. latissima	San Diego Co.	Purer 1942
U. prolifera	Tijuana Est.	this study (see p.76)
E. salina	San Diego Co.	Purer 1942
E. tubulosa	San Diego Co.	Purer 1942
Tribophyta (yellow-green algae)		
Vaucheria sp.	Mission Bay	Stewart 1991

BACILLARIOPHYCEAE

Diatoms are the most common members of many microphytobenthic floras, often substantially outnumbering cyanobacteria and green algae in terms of species richness and sheer abundance (see discussion in Chapter V). Pennate forms dominate the benthic flora but centric diatoms (e.g., *Melosira*) may also be present either as permanent residents of surface sediments or as settled phytoplankton cells (Admiraal 1984, Round et al. 1990).
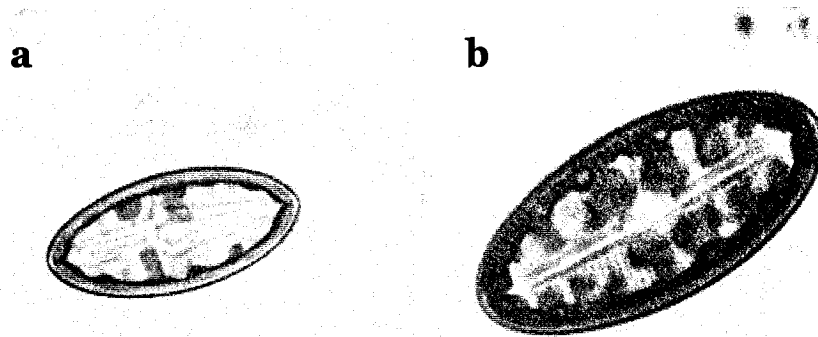
Cocconeis sp(p).

a   b

Figure A1.22. *Cocconeis* specimens from Mission Bay salt marsh sediments. (a) length of cell=38.1 μm. (b) Specimen collected from lightly vegetated salt marsh in Mission Bay during October 2003.

Amphora

A number of *Amphora* species are listed for the local flora: Zedler (1982) lists 3 species for Tijuana Estuary and Wilson (1980) records 17 taxa for Mugu Lagoon in Los Angeles County.

Figure A1.23. *Amphora* specimen from culture; originally isolated from Mission May sediments; bar=10 μm.

Entomoneis

Entomoneis is a diverse genus of pennate diatoms, usually from saline waters (Round et al. 1990). Twisted valves render the organisms bi-lobate in appearance.

Figure A1.24. *Entomoneis* collected from a cyanobacterial mat in Mission Bay salt marsh, October 2003.; bar=10 μm.

Diploneis

Figure A1.25. *Diploneis* collected from Mission Bay vegetated marsh during summer 2004. This is the only observed specimen of this taxon; bar = 10 μm.

Unknown chain diatom

Tube-dwelling chain diatoms such as the specimens below have been observed occasionally in wetland sediments.
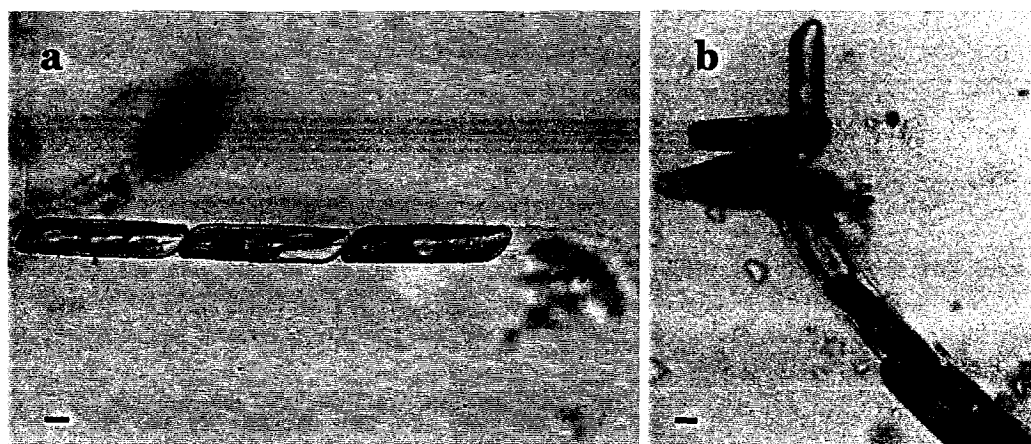

Figure A1.26. Chained diatoms. (a) Specimen collected from lightly vegetated salt marsh, October 2003. Bar=10 μm. (b) Specimen collected during July 2004 from vegetated salt marsh composed of *Salicornia, Batis* and *Triglochin*; bar = 10 μm.
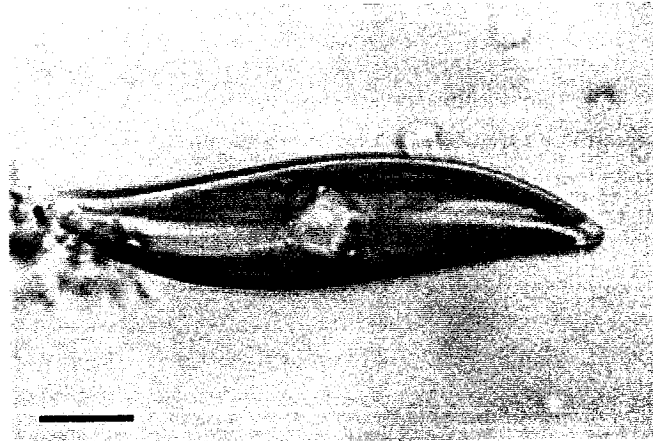
Pleurosigma

Figure A1.27. *Pleurosigma*, collected from *Salicornia/Batis* salt marsh in Mission Bay during summer 2004; bar=10 μm.

Gyrosigma

Figure A1.28. *Gyrosigma*, collected from Tijuana Estuary. No scale available. Organism above *Gyrosigma* is *Pleurosigma*.

Navicula spp.

Navicula is a large genus of pennate diatoms frequently reported from many wetlands worldwide (Peletier 1996). Zedler (1982) lists three species in the local flora and Wilson (1980) included 22 taxa in his work at Mugu Lagoon.
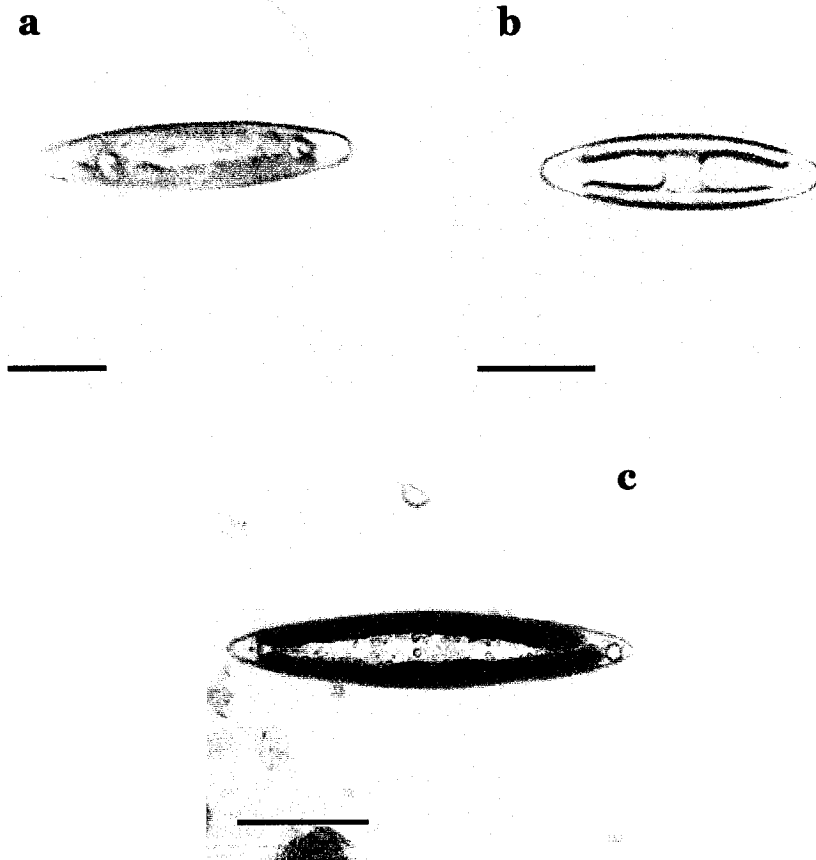
a

b

c

Figure A1.29. *Navicula* spp. (a, b) Naviculoid diatoms from cyanobacterial mats in salt marsh habitat in Mission Bay (collected October 2003 and April 2004 respectively). (a) Length=33.3 μm; bar=10 μm. (b) Length ~24 μm; bar=10 μm. (c) Large *Navicula* collected during summer 2004 from mixed halophyte salt marsh in Mission Bay; cell length=156 μm; bar=50 μm.

Cylindrotheca

Cylindrotheca has been observed from both Mission Bay and Tijuana Estuary sediments. Cells are motile.
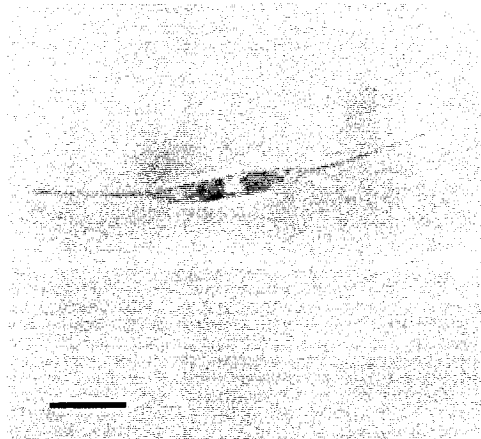
Figure A1.30. This organism was collected from a cyanobacterial mat in the Kendall-Frost Reserve, Mission Bay during April 2004. Total cell length=53.5 μm; bar=10 μm.

Melosira

Melosira, though a centric diatom, is found in the benthos of both saline and freshwater ecosystems (Admiraal 1984).
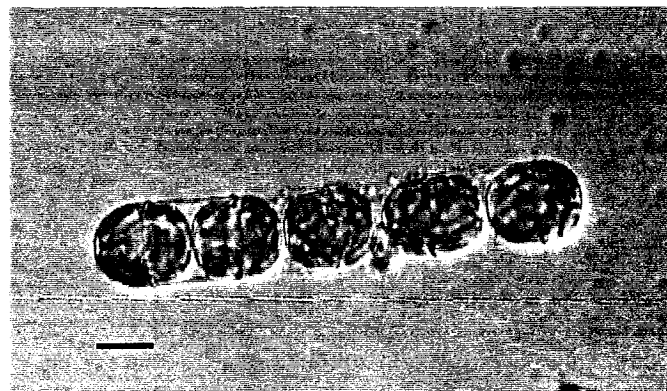
Figure A1.31. *Melosira* collected from Mission Bay during April 2002; bar=10 μm.
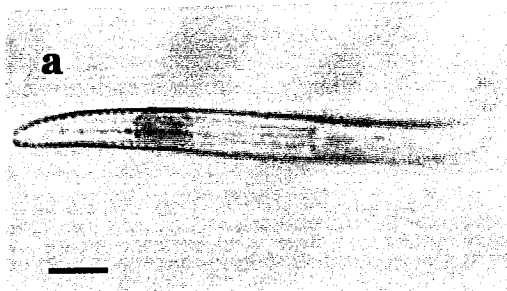
Nitzschia

Figure A1.32. *Nitzschia* sp. collected from salt marsh cyanobacterial mat, April 2004. Total cell length=81 μm; bar=10 μm.
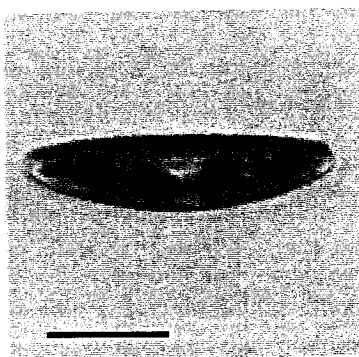
Cymbella

Figure A1.33. *Cymbella* collected during October 2003 from lightly vegetated salt marsh; bar=10 μm.

Unidentified pennate diatoms
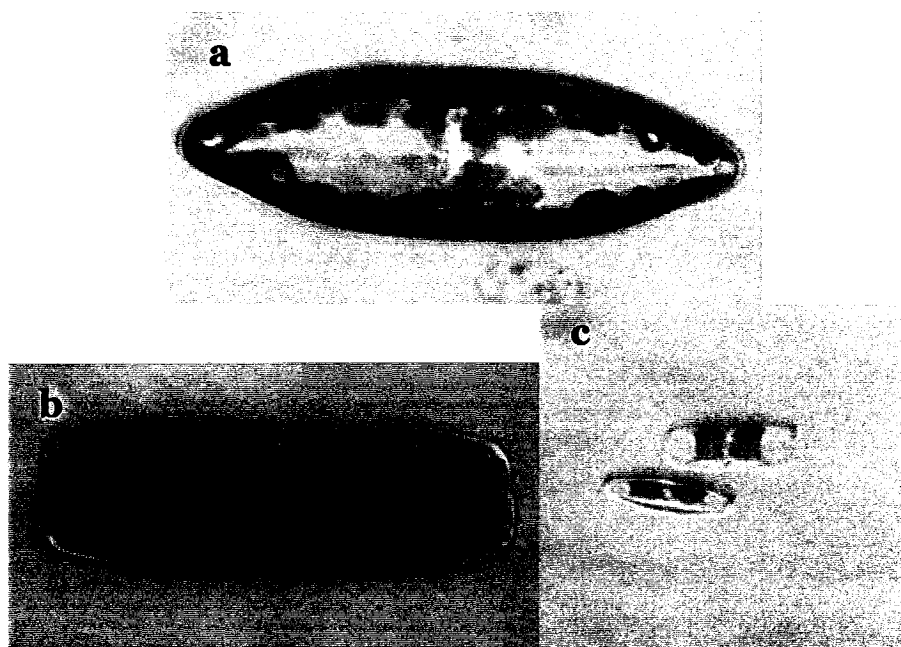
Figure A1.34. Various unidentified diatoms collected from Mission Bay sediments. (a) Diatom resembling the freshwater *Stauroneis* (Round et al. 1990), collected in October 2003 from lightly vegetated salt marsh. (b) Large pennate diatom in girdle view, obtained from salt marsh in October 2003; bar= 10 μm. (c) Two cells grown in laboratory culture.

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The material in this appendix was prepared for future publication and the dissertation author is the principle investigator and author.

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Appendix 2.

HPLC-based analysis of sediment-associated pigments in

coastal marine wetlands

Introduction

Many photosynthetic bacteria and eukaryotic algae inhabiting the benthic environments of marine wetlands are microscopic forms dispersed among the inorganic and organic particles composing coastal sediments. Because of such distribution, elucidation of the composition and diversity present in these communities by traditional microscopy can be a time-consuming task subject to a relatively high degree of imprecision. A number of molecular and biochemical techniques are available for the determination of community structure via elucidations of genetic diversity or by analysis of photosynthetic pigment abundances. Each of these techniques possesses its own technical challenges, taxonomic limitations, and difficulties in interpretation, but they also provide researchers the advantages of increased precision in determination of community composition, the ability to detect rare taxa, and in the case of molecular techniques, the ability to distinguish between morphologically similar but genetically un-related organisms. Here I discuss pigment-based approaches to the determination of benthic microphototroph community composition in marine benthic environments.

All phototrophic organisms possess photosynthetic pigments such as chlorophylls, carotenes (hydrocarbon carotenoids), xanthophylls (oxygenated carotenoids) and biliproteins (e.g., phycoerythrin) involved in the collection of light energy. These pigments are incorporated into the internal thylakoid membranes of plastids in eukaryotic algae or are found imbedded in the cell membranes (or internal structures called chlorosomes) of the anoxygenic photobacteria (Kondratieva et al. 1992). Pigments function in both the harvesting of light for photosynthesis and in photoprotective functions (Falkowski and LaRoche 1991), the latter of which is

absolutely crucial for the success of the photosynthetic reactions (Krinsky 1979). All species of algae, photosynthetic bacteria, and vascular plants simultaneously possess several different kinds of pigments, but chlorophyll a occurs ubiquitously in the algae, cyanobacteria, and vascular plants (all oxygenic producers). Chlorophyll a and bacteriochlorophyll a serve at the reaction centers of membrane photosystems (PSI, PSII) involved in the first steps of the photosynthetic process (Cogdell 1985). Peripheral light harvesting complexes contain both additional chlorophyll and carotenoid molecules associated with proteins (Cogdell 1985).

Various photopigments can be used (with caution) as diagnostic markers of particular algal groups in the marine environment (Jeffrey 1997). A few photopigments, such as peridinin, are limited in occurrence to single high-level taxonomic groups, such as division or classes. Many other pigments are shared between a few major groups (e.g., diatoxanthin, diadinoxanthin) and a few are nearly ubiquitous in distribution (e.g., β-carotene); all of these would be poor indicators of the abundance of a particular group because of their widespread distribution. However, given some background knowledge about the kinds of functional groups present in a given marine habitat, certain pigments can be used as to mark selected taxa when other taxa containing that pigment are presumed to be absent.

For the most frequently encountered phototrophs in the sediments of coastal marine habitats, the major carotenoids fucoxanthin and zeaxanthin can be used to indicate the presence and biomass of diatoms and cyanobacteria respectively (Pinckney et al. 1995). Myxoxanthophyll, oscilloxanthin, or echinenone concentrations can also be used as estimates of benthic cyanobacterial biomass (Kirk 1994, van den Hoek 1995, Camacho and de Wit 2003), although the latter, like zeaxanthin, is not completely exclusive to the cyanobacteria (Jeffrey et al. 1997a).

Lutein or chlorophyll b, major pigments of green algae and land plants, may also be useful markers for the presence of these organisms in wetlands (Buffan-Dubau and Carman 2000), although chlorophyll b is also found in euglenoids (Fiksdahl and Liaaen-Jensen 1988), a group occasionally observed in wetland sediments. Peridinin (or dinoxanthin) can serve as a marker for dinoflagellates (Buffan-Dubau and Carman 2000). For the major groups of anoxygenic photosynthetic bacteria present in marine waters, bacteriochlorophylls a and c, d, e show promise as indicators of the purple (sulfur and non-sulfur) and green (Chlorobiaceae and Chloroflexaceae) phototrophic bacteria respectively (Madigan 1988, Pinckney et al. 1995, Imhoff 1999).

Routine quantification of pigments in solvent extracts is performed by fluorometric or spectrophotometric techniques. However, these methods (while faster and cheaper than HPLC) suffer from several shortcomings. First, separation by HPLC allows for more precise quantification of chlorophyll a, for example, than spectrophotometry or fluorescence detection (Pinckney et al. 1994) and alleviates the concern that absorption readings at a certain wavelength might be due to more than a single pigment (Millie et al. 1993). Furthermore, pigments of similar absorption spectra (e.g., many algal carotenoids) and closely related isomers of single pigments can be carefully separated and quantified by HPLC but cannot be readily quantified by spectrophotometry.

Although phytoplankton biologists routinely separate algal and bacterial pigments by HPLC to elucidate the structure of plankton communities with great analytical care (Jeffrey et al. 1997b), pigment work in sedimentary material is less precisely developed and poses several unique challenges largely unseen in plankton work. For example, sediments may contain large amounts of degraded cellular material that are difficult to ascribe to any given taxon and which lead to separations

replete with minor unidentified components. Moreover, pigment extraction

techniques have not been rigorously examined in benthic microphototrophs (but see

Buffan-Dubau and Carman 2000), so the best preservation techniques and extraction

solvents for organisms common to the marine benthos are not well known. Finally,

the moisture content of sediments can vary considerably from sample to sample,

posing a minor challenge for the maintenance of constant extraction conditions.

In this appendix I describe the techniques employed in my dissertation work

for the analysis of pigments via HPLC from sediment phototroph communities from

southern California wetlands. Specifically, I present the following data: (1) a

description of the bi-phasic gradients used in reverse-phase separations and elution

times of major algal and bacterial pigments along these gradients, (2) the pigment

composition of algal and plant reference material used to help identify unknown

pigments in sediments, (3) analysis of the efficiency of pigment extraction in 90%

acetone, and (4) pigment-specific calibration equations. I also discuss the various

challenges associated with pigment work in sediments, including the use of pigment

concentrations as estimates of phototroph biomass.

Materials and methods

Pigment extraction. Sediments were obtained in the field at low tide from

coastal wetlands in Mission Bay and Tijuana Estuary in southern California during

2000-2004. Cores of surface sediment to 2-10 mm depth were collected with a plastic

syringe of (usually) 8.5 mm diameter and were stored in sealed glass containers (see

Chapters II-IV for specific sampling locations and techniques). In conjunction with

many of the sediment collections made for pigment work, a corresponding core of

equivalent dimensions (situated usually within a few cm of the pigment core) was also collected and placed in a pre-weighed glass container to estimate sediment moisture retention for pigment extractions. Sediment collected for pigment work was (usually) stored at -80°C and material for the estimation of the water capacity of sediments was stored at -20°C.

Sediment for estimation of water capacity was defrosted, weighed and subsequently dried at ~50°C to ~60°C (for at least 12 hr) before a final mass was determined. Water capacity was measured as the mass loss between wet and dry sediment. Core-specific estimates of water content were used to estimate the quantity of 100% acetone needed to create an extraction solution of 90% acetone. Sediment material was extracted for 24±1 hr in the dark on ice (usually 0-4°C). Samples were centrifuged and then extracts were purified by filtration through cotton set inside a glass Pasteur pipettes (Goericke 2002). To test the efficiency of extraction using the above procedure, an additional 5.0 ml of 90% acetone was added to four selected samples after supernatant had been removed from extraction containers. Re-extraction was again performed on ice for 24 hr. Re-extracts were analyzed on gradient G (see below) and initial (P_I) and re-extract (P_R) concentrations of chlorophyll a were determined; extract efficiency is expressed as E= $P_I/(P_I + P_R)$ x 100%.

Pigment separation. Chlorophylls and carotenoids were separated via reverse-phase HPLC according to one of five bi-phasic gradients developed by R. Goericke (Gradient A, B) or Chris Janousek (Gradients F, G, H). Gradients F and H were derived from the published techniques of Brotas and Plante-Cuny (1996) and gradient G was modeled after Goericke (2002). Solution composition throughout the duration of each gradient is enumerated in Table A2.1. Gradients A, B, F, and H

utilized an Adsorbosphere® C_{18} HS reverse-phase column (3 μm diameter stationary phase) coupled to a C_{18} HS guard column and 0.5 μm prefilter. An Adsorbosphere® C_{18} column (of 15 cm length, 5 μm stationary phase) was used with the same pre-column and prefilter as above for gradient G separations. Dissolved oxygen in mobile phases was removed for many of the separations by sparging with either helium or nitrogen gas.

Two independent HPLC systems were utilized for pigment separation. HPLC system *1* consisted of pumps, and a photo diode array detector (absorption spectra from ~350 to ~800 nm were generated) and was used to separate early Tijuana Estuary sediments (Sept. 2000; see Chapter III) via gradient A. Extracts (300 μl of a 12:5 solution of extract and water) were manually injected into the system.

On HPLC system *2*, mobile phases were delivered by a 600E pump and to a variable wavelength (Thermo Separation Products) and Waters® 470 fluorescence detector. Extracts (100 μl) were automatically injected by an autosampler. On HPLC system *2*, pigment abundances were determined by absorption (450 nm for gradients E, F, and H; 770 nm for gradient G) and by fluorescence (excitation at 430 nm, emission at 674 nm; all gradients). Detector outputs (in μV or mV) were converted to mass (ng) of pigment using pigment-specific calibrations generated independently with purified pigment material. During 2001-2005, relationships between detector output and pigment mass were generated twice for fucoxanthin, lutein, zeaxanthin, and peridinin and once for chlorophyll a, bacteriochlorophyll a, and bacteriopheophytin a. Purified carotenoids were obtained from DHI Water and Environment (Hoersholm, Denmark). Bacteriochlorophyll a was derived from *Pseudorhodomonas sphaeroides* (Sigma-Aldrich, Inc.) and bacteriopheophytin a was generated in the lab from bacteriochlorophyll a stock solution by treatment with 0.1 N

Table A2.1. Solvent schedule for biphasic gradients used in sediment pigment research. See text for solvent compositions. All gradients utilized a linear transition between each new solvent combination.

Gradient A

Time (min)	% phase 1	% phase 2
0	100	0
2	75	25
30	50	50
42	5	95
43	5	95
45	100	0

Gradient B

Time (min)	% phase 1	% phase 2
0	100	0
2	75	25
30	50	50
42	5	95
45	5	95
48	100	0
50	100	0

Gradient F

Time (min)	% phase 1	% phase 2
0	100	0
30	0	100
37	0	100
40	100	0

Gradient H

Time (min)	% phase 1	% phase 2
0	70	30
30	0	100
37	0	100
40	70	30

Gradient G

Time (min)	% phase 1	% phase 2
0	80	20
1	50	50
10	0	100
14	0	100
16	80	20

hydrochloric acid in 90% acetone.

Pigment identification. Pigments extracted from unialgal material were identified by PDA-generated absorption spectra (system *1*), via the assistance of R. Goericke, and by use of the following references: Volkman et al. (1993), Tanaka et al. (1974), Anderson et al. (1991), Knoetzel and Rensing 1990, Suda et al. (1993), and Jeffrey and Wright (1997).

Results and discussion

Gradients and pigment elution. Gradient A, developed by R. Goericke in 2000 (and gradient B, a slight variation of A), employed a 40 min bi-phasic gradient that adequately separated lutein and zeaxanthin (Table A2.2). Fucoxanthin (found in diatoms) however, co-eluted with neoxanthin (found in green algae and plants), and separation of myxoxanthophyll (cyanobacteria) and violaxanthin (green algae/plants) was also incomplete (a disadvantage only if green algal/plant and cyanobacterial pigments dominate sediment extracts). Diadinoxanthin and diatoxanthin did not elute with any other major pigments tested. Chlorophyll a was immediately followed by echinenone (usually from cyanobacteria) but could be identified by its characteristic absorption spectrum and could usually be manually separated from chlorophyll a. Gradient E was an adapted form of gradient A used on system *2*.

Gradients F and H were developed from the gradient used in Brotas and Plante-Cuny (1996) to alleviate the co-elution of neoxanthin with fucoxanthin and violaxanthin with myxoxanthophyll and to eliminate use of acetonitrile, a highly toxic organic solvent. With these gradients (Table A2.1) lutein and zeaxanthin were again adequately separated (~0.3 min gap) and a good separation of the

Table A2.2. Elution schedule for pigments on gradient A and B separations developed by R. Goericke in 2000. For gradient B, mean (± 1 S.D.) are given.

Pigment	Elution (min)	
	Gradient A	Gradient B
chlorophyll $c_{1,2,\text{ and/or }3}$	5.6	6.8±0.15
peridinin	8.9	10.63±0.85
fucoxanthin	11.1	12.19±0.75
neoxanthin	~11.2	12.25±1.03
violaxanthin	14.7	15.92±1.49
myxoxanthophyll	14.7	15.18±1.32
diadinoxanthin	18.2	19.60±1.08
vaucheriaxanthin-ester	ND	19.41±0.20
diatoxanthin	23.8	24.43±1.32
lutein	25.2	25.69±1.41
zeaxanthin	25.6	26.38±1.45
canthaxanthin	29.7	31.02
bacteriochlorophyll a		ND
chlorophyll b	37.1	39.19±0.68
chlorophyll a	39.2	41.25±0.42
α, γ carotenes		45.03±0.21
β carotene		45.52±0.25
Gradient duration (min)	45	50
Column	C_{18} HS, 3µm, 10cm	

neoxanthin/fucoxanthin and myxoxanthophyll/violaxanthin pairs was achieved (Table A2.3). α and γ carotenes and the very polar chlorophyll $c_{1,2,3}$ that eluted early were not separated from each other (Brotas and Plante-Cuny 1996; Table A2.3). Unfortunately, vaucheriaxanthin-ester (a good marker for the benthic tribophyte *Vaucheria* and planktonic eustigmatophytes) and alloxanthin (a good marker for cryptomonads) were not adequately resolved. However, most other major pigments present in green algae, diatoms, dinoflagellates, and cyanobacteria were adequately separated. Crocoxanthin or monadoxanthin may be alternative markers for the Cryptophyceae with these gradients.

In gradient G, bacteriochlorophyll a eluted from the column at approximately 12.0-12.2 min and was followed by chlorophyll a and bacteriopheophytin a at 13.7 min (Table A2.4). Analysis of sediment material from Mission Bay and Tijuana Estuary sediment suggested the presence of several other unknown pigments showing at least minimal absorption at 770 nm (unk 1-6 in Table A2.4). In order to determine potential candidates for bacteriochlorophyll b among these unknowns, several extracts were separated again with absorption at 791 nm (Hoff and Amesz 1991). Based on increased absorption at 791 nm relative to 770 nm, two unknown pigments may be bacteriochlorophyll b (or bacteriochlorophyll b-derived pigments): unk 2 and unk 5 (Table A2.5). Based on fluorescence detection, unk 5 is believed to be chlorophyll a. Thus, unk 2 is a candidate pigment for bacteriochlorophyll b but this should be confirmed by further chromatography (including purification of the pigment itself) and microscopy to verify the presence of bacteriochlorophyll b-containing organisms in southern California's marine wetland sediments.

Purified pigments of various concentrations were prepared by dilution in 90% acetone and were quantified on system 2 to relate detector outputs to known pigment

Table A2.3. Elution schedule for pigments on gradient F and H separations. Pigments were detected by absorption at 450 nm except for chlorophyll a which was detected by fluorescense (exitation: 430 nm, emmission: 674 nm). ND= no data or not determined.

Pigment	Elution (min)		
	Brotas and Plante-Cuny	Gradient F	Gradient H
chlorophyll $c_{1,2, \text{ and/or } 3}$	?8.5	ND	?3.1
peridinin	ND	ND	4.1
fucoxanthin	10.7	12.5	6.2
neoxanthin	11.6	13.4	7.6
violaxanthin	13.3	15.1	10.0
dinoxanthin	ND	ND	11.1
myxoxanthophyll	ND	ND	?~11.7
diadinoxanthin	14.3	17.0	12.6
neofucoxanthin	14.6	ND	ND
vaucheriaxanthin-ester	ND	? 18.9	15.0
alloxanthin	15.5	ND	15.0
monadoxanthin	ND	ND	?15.7
diatoxanthin	15.9	19.6	16.0
lutein	17.2	20.1	16.6
zeaxanthin	17.6	20.4	17.0
chlorophyll b	19.7	25.7	23.3
crocoxanthin	ND	ND	25.5
chlorophyll a	21.5	29.2	27.2
chlorophyll a'	22.0	ND	28.2
α, γ carotene	ND	ND	34.2
β carotene	26.3, 26.8	ND	34.6
Gradient duration (min)	34	40	40
Column (stationary phase dian	C_{18} HL (5μm)	C_{18} HS (5μm)	

Table A2.4. Elution schedule for pigments on gradient G separations. Pigments were detected by absorption at 770 nm except for chlorophyll a which was detected by fluorescence (exitation: 430 nm, emmission: 674 nm). Unk 2 may be bacteriochlorophyll b. ND= no data.

	Elution (min)	
Pigment	Goericke (2002)	Gradient G
unk 1	ND	10.6
unk 2	ND	10.9
unk 3	ND	11.6
unk 4	ND	11.9
bacteriochlorophyll a	6.6	12.2
unk 5	ND	13.0
chlorophyll a	ND	~13.0
bacteriophaeophytin a	ND	13.7
unk 6	ND	14.4
Gradient duration (min)	14	16
Column	C_{18} HIS, 3 µm, 15 cm	C_{18} HS, 5 µm, 15 cm

Table A2.5. The search for bacteriochlorophyll b (bchl b). Absorption of extracts of field sediments were measured at 770 nm (for bacteriochlorophyll a and derivatives) and subsequently measured at 791 nm (near absorption maximum of bacteriochlorophyll b; Hoff and Amesz 1991). Ratios of absorption (791 nm/770 nm) > 1 indicate candidates for bacteriochlorophyll b. All elution data are from Tijuana estuary, April 2002 sediments (see Chapter III); ND= no data

791 nm/770 nm absorption

Pigment extract	unk 1	unk 2	unk 3	unk 4	bchl a	unk 5	bph a	unk 6
MBAY.06/02.32	0.35	1.15	0.77	0.78	0.33	0.99	0.08	ND
MBAY.06.02.03	0.30	1.18	ND	ND	0.39	1.13	ND	ND

	unk 1	unk 2	unk 3	unk 4	bchl a	unk 5	bph a	unk 6
Mean elution (min)	10.50	10.87	11.50	11.78	12.10	12.91	13.61	14.31
S.D. elution time (n)	0.04 (14)	0.05 (8)	0.05 (12)	0.06 (7)	0.05 (19)	0.06 (9)	0.06 (18)	0.08 (4)

concentrations (Table A2.6). Very precise (all $r^2 > 0.99$) linear relationships were seen in every case. Since visual inspection suggested that all relationships were linear across all concentrations tested, extrapolation for any given pigment should not be problematic within the natural limits of a given detector. All linear regressions based solely on the pigment concentrations used had a slight negative or positive intersection with the y-axis (Table A2.6). For most pigment concentrations, errors generally had a negligible influence on the accuracy of pigment ratios or on the determination of functional diversity, although zeax/fuco and functional diversity were slightly inflated at low biomass upon use of original relationships. Thus, alternative detector-mass relationships (forced through the origin) were generated for each pigment and used in place of original relationships for many separations (Tijuana Estuary data generated on system 2 from April 2000 to April 2003 [Chapter III], culture pigment concentrations from spring 2005 [Chapter IV] and all bacteriochlorophyll a concentrations in Chapters II and IV).

Pigment composition of reference material. *Thalassiosira weisflogii* (Figure A2.1), *Dunaliella tertiolecta* (Figure A2.2), *Arthrospira platensis* (Figure A2.3), *Lingulodinium polyedrum* (Figure A2.4), and *Nannochloropsis oculata* (Figure A2.5) were regularly used as reference taxa to compared known pigments with the elution times of unknown pigments in sediment material (Figure A2.6). *Thalassiosira* and *Dunaliella* cultures provided consistent, reliable separations with little degraded material. Leaves from mint (*Mentha* sp., Lamiaceae) and peppermint (*Mentha x piperita*) were also routinely used (pigment composition similar to that of *Dunaliella*; data not shown). Additionally *Chroomonas* sp. (Cryptophyceae) cultures were used to check for co-elution of pigments with other major taxa during the development of gradient H (Figure A2.6). Reference material was analyzed

Table A2.6. Pigment detector-mass relationships for selected microphytobenthic pigments (HPLC machine 2). "fl"=fluorescence detector; "vwd"=variable wavelength detector. "Alternative calibrations" were forced through the origin (i.e., 0 ng pigment must equal 0 mV). ND= not determined.

| Pigment	Taxonomic distribution	Calibration on...	For	λ(nm)	n	r^2	Calibration	Alternative calibration
Chlorophyll a	oxygenic phototrophs	Fall 2001	fl	430/674	7	0.997	ng: 0.0009195mV-0.503	ng: 0.0009183mV
Chlorophyll a		Fall 2001	vwd	450	8	0.996	ng: 0.0002531mV+0.329	ng: 0.0002534mV
Bchl a	anoxy. phototr.	July 2004	vwd	770	5	0.999	ng: 0.0001366mV+4.971	ng: 0.0008648mV
Fucoxanthin	diatoms	Fall 2001	vwd	450	7	0.998	ng: 0.0000185mV+0.652	ng: 0.0000198mV
Fucoxanthin		June 2003	vwd	450	4	>0.999	ng: 0.0000449mV-0.109	ng: 0.0000447mV
Zeaxanthin	cyanobacteria	Fall 2001	vwd	450	6	0.998	ng: 0.0000196mV+0.538	ng: 0.0000220mV
Zeaxanthin		June 2003	vwd	450	5	0.996	ng: 0.0000235mV-0.091	ng: 0.0000233mV
Lutein	green algae/plant detritus	Fall 2001	vwd	450	4	>0.999	ng: 0.0000219mV+0.088	ng: 0.0000201mV
Lutein		June 2003	vwd	450	4	0.993	ng: 0.0000225mV-0.361	ng: 0.0000237mV
Peridinin	dinoflagellates	Fall 2001	vwd	450	5	0.994	ng: 0.0000459mV-0.693	ND
Peridinin		June 2003	vwd	450	4	>0.999	ng: 0.0000428mV-0.370	ng: 0.0000423mV
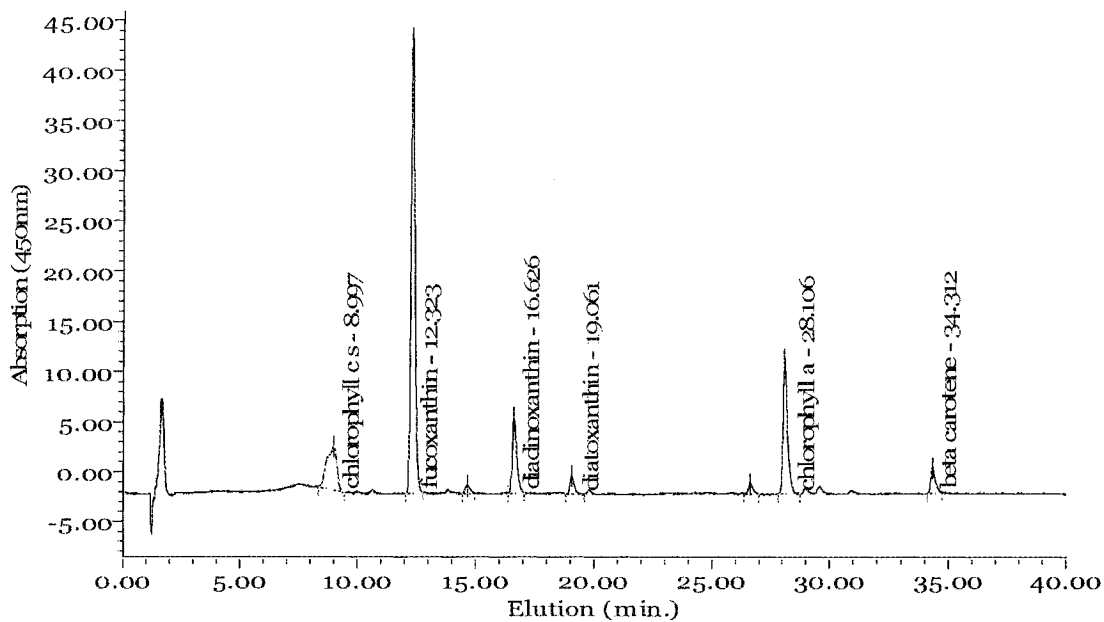
Figure A2.1. Separation of pigments from the pelagic diatom *Thalassiosira weisflogii* (via gradient F), used as a standard throughout this dissertation. See Latasa (1995) for the pigment composition of *T. weissflogii*.
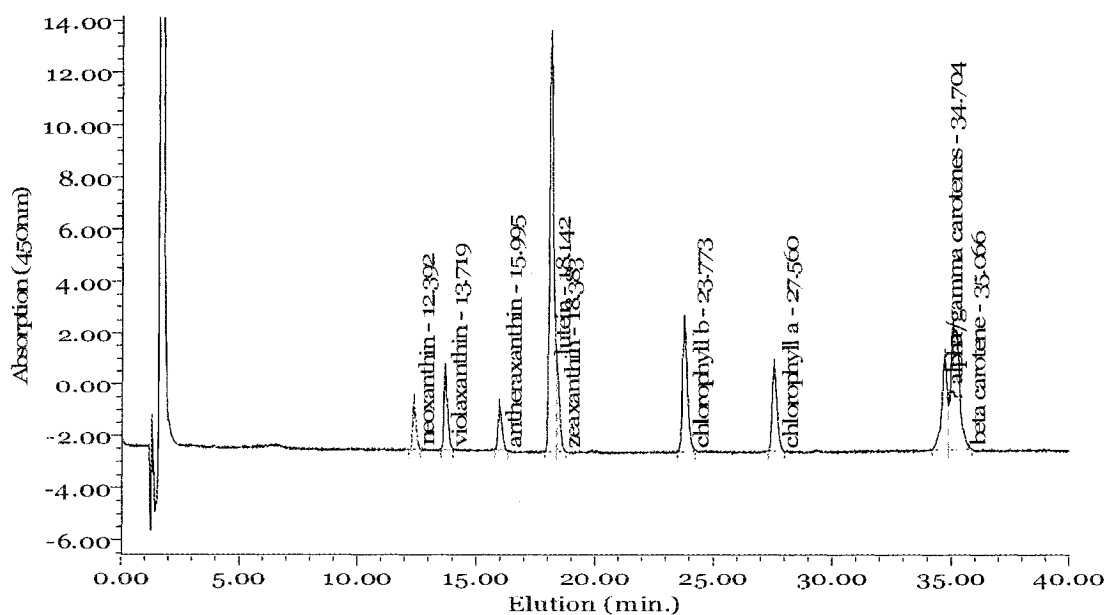

Figure A2.2. Separation of pigments from the alga *Dunaliella tertiolecta* (Chlorophyta) via gradient F. Pigment reference: Volkman et al. (1993).
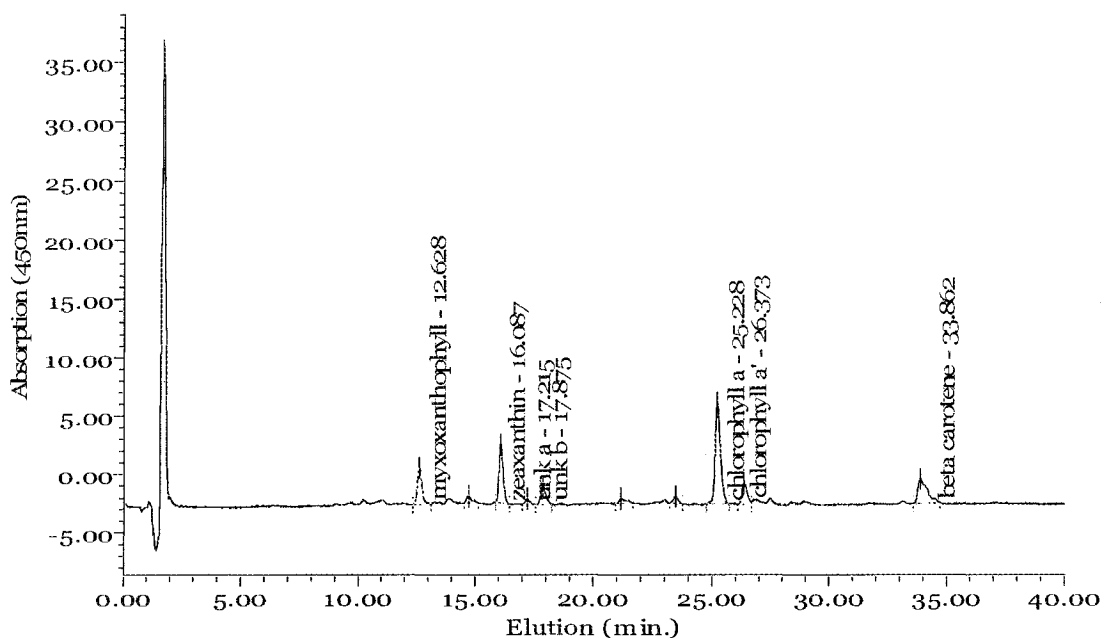
Figure A2.3. Pigments of commercially available *Arthrospira platensis*, used to confirm elution times of cyanobacteria-derived pigments in sediments. *A. platensis* is also believed to possess lutein, echinenone, β-cryptoxanthin, hydroxyechinenone, euglenanone, α-carotene and possibly additional carotenoids (Tanaka et al. 1974, Anderson et al. 1991). Separation on gradient F.
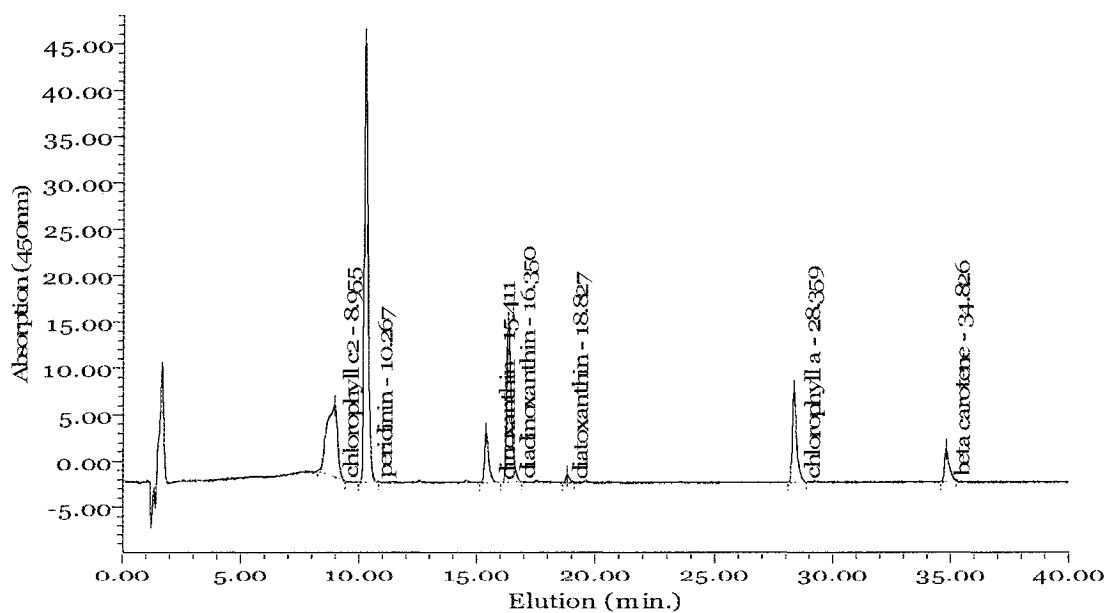
Figure A2.4. Pigments of the dinoflagellate *Lingulodinium polyedrum*, separated on gradient F. Additional reference: Knoetzel and Rensing (1990).
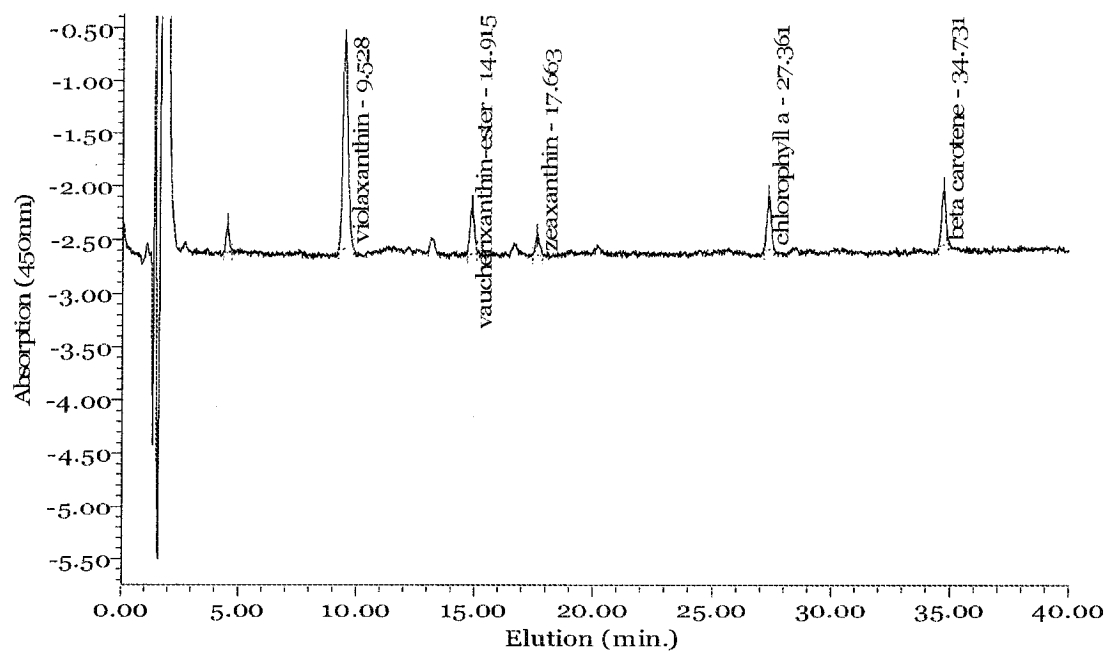
Figure A2.5. Pigments of *Nannochloropsis oculata* (Eustigmatophyceae) separated on gradient H (Volkman et al. 1993, Suda et al. 2002). Vaucheriaxanthin-ester is also found in the sediment-dwelling *Vaucheria* (Tribophyceae).
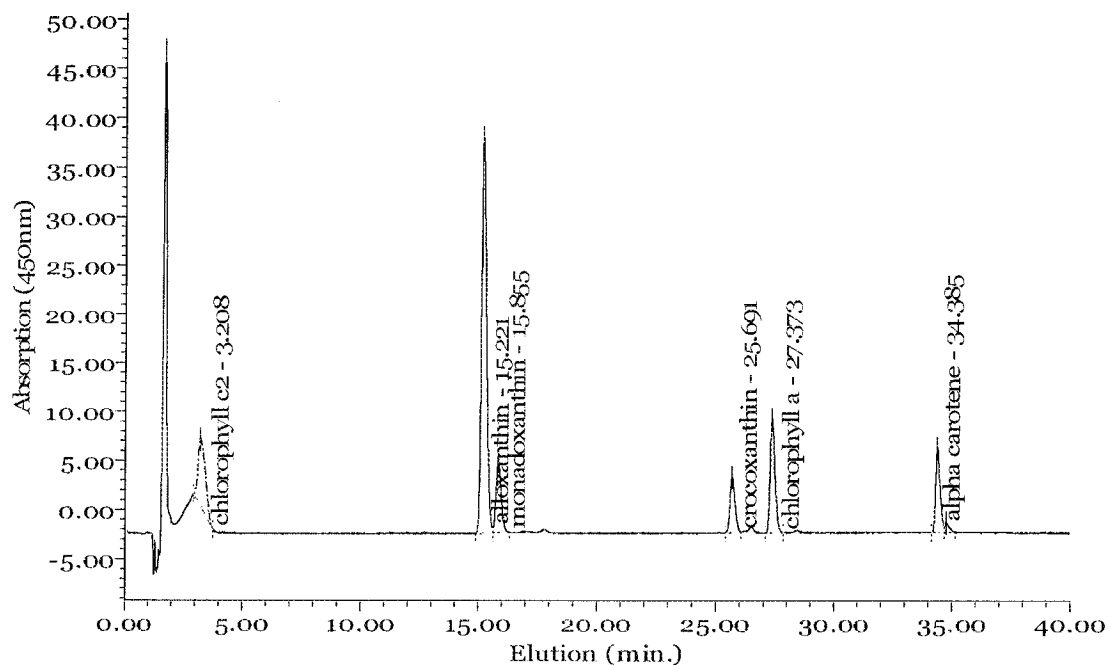

Figure A2.6. Pigments of *Chroomonas* sp. (Cryptophyceae). See Jeffrey and Wright (1997) for the pigment composition of *Chroomonas salina*.

periodically with sediment samples to aid in the identification of pigments in sediment material (e.g., Figure A2.7). Because of day to day shifts in elution times (up to 1-2 min), or drifting elution times from sample to sample over the course of a day, it was necessary to include these reference cultures.

Extraction efficiency. Extraction of lipid-soluble photosynthetic pigments is possible with a variety of solvent combinations (Jeffrey et al. 1997 book). The efficiency of various extraction techniques for various phytoplankton species has been rigorously tested by (in Jeffrey et al. 1997). Less is known about the efficiency of extraction of sediment-dwelling phototrophs. However, Buffan-Dubau and Carman (2000) suggest freeze drying followed by extraction in 100% acetone as the most effective technique. In this work, 90% acetone was used to minimize the activity of the enzyme chlorophyllase which leads to degradation of chlorophyll a (Rüdiger and Schoch 1988). A test of the extraction efficiency by comparing initial and re-extracts of sediment material suggested that at least 90% (mean=91%, n=4) of chlorophyll a was released from the sediment and benthic cells during the first initial 24 hr extraction period using 90% acetone. At least some of the chlorophyll a present in the second extraction round was probably contained within the wet sediment remaining after taking up initial supernatant, suggesting possibly an even greater efficiency in initial extraction. However, re-extraction was not attempted in other solvents nor tested on other major pigments, so these data remain preliminary. It is known, for example, that pigments from certain algal taxa may be difficult to extract (Wright et al. 1997), so a single extraction solvent could favor a given algal group in terms of extraction efficiency.
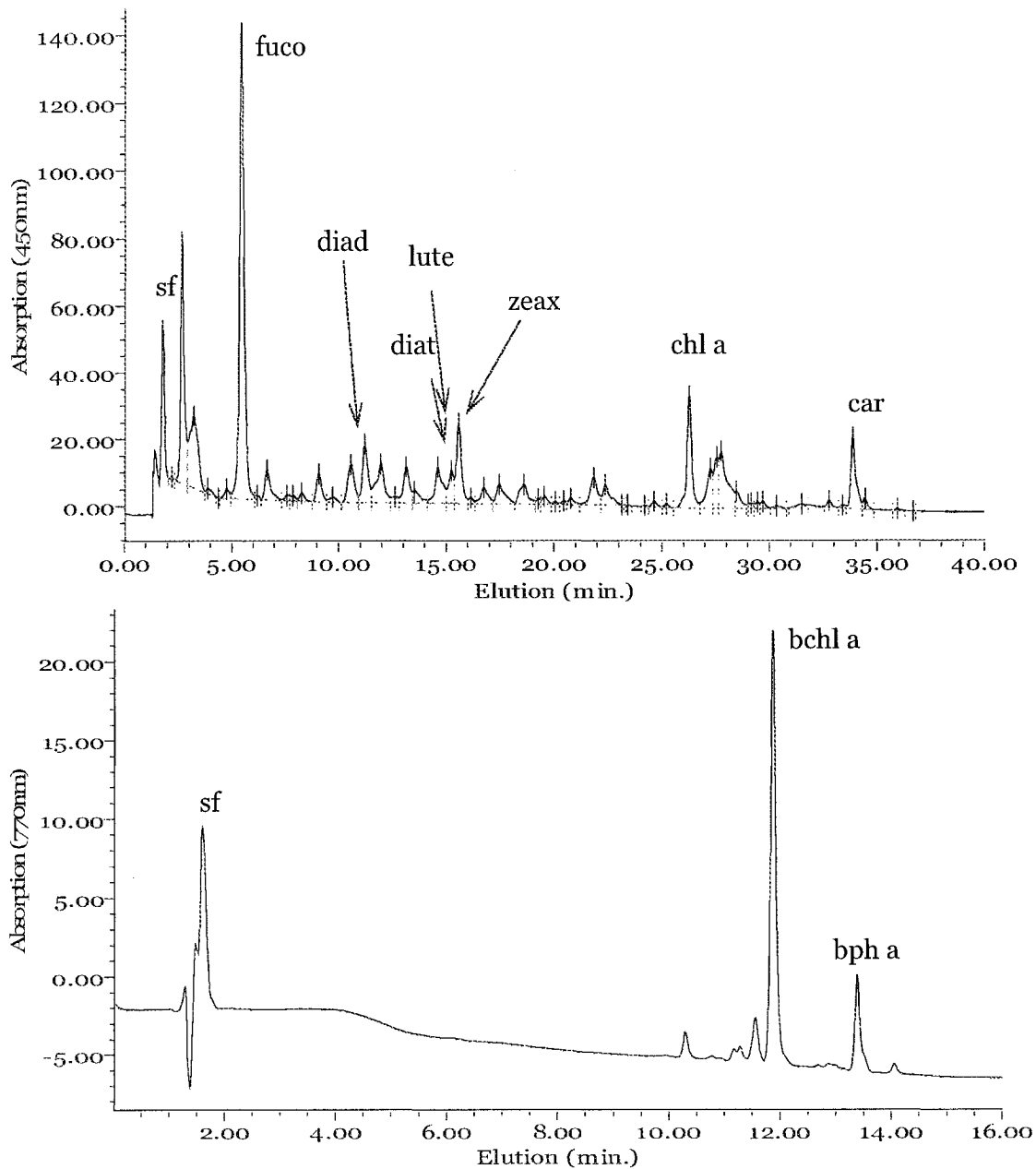
Figure A2.7. Separation of sediment pigments via (a) gradient H and (b) gradient G (sediment collected during summer 2004, Mission Bay salt marsh). sf=solvent front, fuco=fucoxanthin, lute=lutein, diad=diadinoxanthin, diat=diatoxanthin, zeax=zeaxanthin, chl a=chlorophyll a, car=carotenes, bchl a=bacteriochlorophyll a, bph a=bacteriopheophytin a.

Sediment-specific pigment considerations. Marine wetland sediments
collected in conjunction with ecological research are usually obtained during periods
of low tide when field sites are much more accessible to field research, but coastal
sediments vary in water retention on short time scales because of diurnal tidal
inundation (or rarely in southern California, because of variation in rain fall). Even
during low tide, water retention in sediments can vary substantially between and
within habitats. For example, the moisture content of sediment collected from
various habitats in Mission Bay during late March 2002 (n=56; see Chapter II) varied
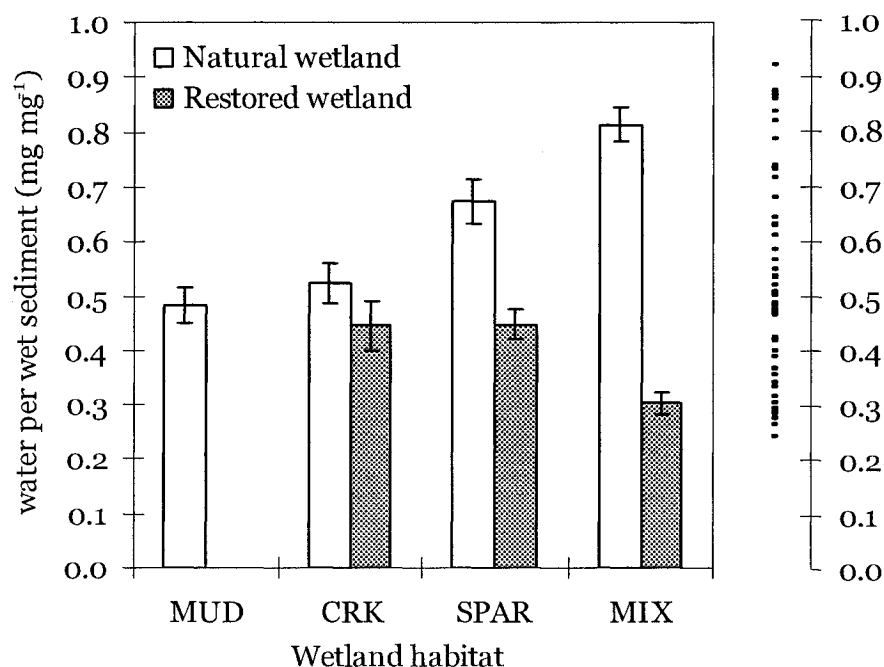from 25% to 92%, a more than 3-fold difference (Figure A2.8).

Figure A2.8. Variability in the water content of sediments collected from various
Mission Bay habitats during late March 2002. (left: means ±1 S.E. according to
habitat type; right: individual sediment communities). MUD=mudflat, CRK=creek-
bank, SPAR=*Spartina* marsh, MIX=mixed halophyte salt marsh.

Because of such variability in the water content of sediment (due to changes in organic matter, sediment grain morphology or intertidal height), it may be desirable to adjust extraction techniques to account for the actual abundance of seawater present in each sediment community, or remove water altogether. Several possible methodologies are suggested. First, collected sediment can be freeze-dried to extract water and then treated with appropriate extraction solvents (Buffan-Dubau and Carman 2000). Second, estimates of seawater present in each core could be obtained by collection of an additional small sediment core (of similar diameter and depth) adjacent to the original collection. The latter sediments are then weighed, dried, and reweighed for an approximation of water capacity which can then be directly utilized to determine the abundance of extraction solution desired for pigment work. This dried sediment might then be further used to estimate sediment organic matter or particle size distribution.

Pigment concentrations are routinely used in oceanographic and limnological work for estimation of the biomass of natural populations of microproducers, but care must be taken in the interpretation of pigment concentrations. This is necessitated by the fact that changes in the ratio of certain key pigments to cellular biomass (e.g. carbon content) can be induced by variation in light levels (Falkowski and LaRoche 1991) or nutrient availability (Geider et al. 1998) in the field. Increases in cellular chlorophyll a concentrations with lower irradiance have been well documented and should be accounted for in studies where chlorophyll a is quantified through the water column or across wetland habitats of dramatically different light regimes. Certain pigments may respond more dramatically to changes in light or nutrient levels than others. For example, zeaxanthin may change little across large variation in irradiance (Kana et al. 1988).

Three approaches will help maximize the utility of pigment concentrations as estimates of population abundance in community ecology. First, selection of pigments that are largely non-responsive (i.e., cellular carbon to pigment ratios change little) to changes in light or nutrient status can be used as taxonomic biomarkers. Second, studies can be conducted among (but not between) habitat areas that are known to be variable in light or nutrient concentrations. However, this is often unfeasible for many important ecological questions. Finally, researchers can use of ratios of pigments that respond in a similar way (direction and magnitude) to light and/or nutrient variation may be one way to confidently characterize community composition.

Taxon-specific variation in the content of cellular pigment concentrations may also occur between individual genera, species, and strains from the same major taxonomic group (Whittle and Casselton 1975, Pennington et al. 1988). These various complex interactions of environment, physiology and phylogeny that influence pigment abundance in natural settings make it impossible to fully predict pigment-biomass relationships in assemblages of phototrophic organisms. Nevertheless, because it is such a powerful technique, ecologists will continue to rely on taxon-specific pigment analyses to study marine communities. Careful consideration of the limitations of the approach and the interpretation of results will obviate the more egregious problems in pigment ecology.

Acknowledgements

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