THE BACTERIAL SULFUR CYCLE OF INTERTIDAL SEDIMENT IN A PACIFIC ESTUARY

by

DAVID JOHN MINTER

A DISSERTATION

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

June 1982

APPROVED: Paul P. Rudy

Prof. Paul P. Rudy, Co-Chair

William R. Sistrom

Prof. William R. Sistrom, Co-Chair

An Abstract of the Dissertation of

David John Minter for the degree of Doctor of Philosophy

in the Department of Biology to be taken June 1982

Title: THE BACTERIAL SULFUR CYCLE OF INTERTIDAL SEDIMENT IN A PACIFIC ESTUARY

Approved:

Prof. Paul P. Rudy, Co-Chair

Prof. William R. Sistrom, Co-Chair

Abundance and activity of certain sulfur cycle microbes were measured at two locations within Coos Bay, Oregon. One location was a typical mudflat having no vascular plants, the other was an atypical sheltered mudflat of higher organic content having vascular plants during the summer. Both locations showed annual periodicity in sulfur cycle activity. Sulfide production and consumption were slightly higher during the warm months (July to December) than the cold months (January to June), sulfur cycle bacteria were most active in late autumn.

Measured rates of sulfide production by anaerobically respiring bacteria (about 10^{-4} moles per square meter per hour) would require the breakdown of as much carbon as that fixed by photosynthesis *in situ*, suggesting that some organic matter is imported.

The rate that volatile sulfur compounds are emitted to the atmosphere from the sediment is as great as the estimated sediment production of sulfide, indicating sources in addition to the sulfide produced beneath the oxygenated surface layer.

The rate of carbon fixation by non-illuminated surface sediment under specified laboratory conditions increases during the months of greatest sulfide flux, this estimate of non-photosynthetic fixation by sulfide consumers is an order of magnitude less than photosynthetic fixation.

Although species of the photosynthetic green and purple bacteria could be isolated from intertidal mud at many Coos Bay locations, they were abundant only at the few locations similar to the high organic content sampling site. The only bacteriochlorophylls measurable in alcohol extracts of sediment were those of purple bacteria at such sites. At the high organic content location bacteriochlorophylls were greatest at two horizons within the sediment. The first horizon, at the sediment surface, contained bacteria growing photosynthetically. The second horizon, at ten centimeters depth, contained bacteria that seemed to be dependent on decomposition of wood fibers remaining from logging operations a decade ago.

During autumn months the purple bacteria of the surface horizon fixed as much carbon as did algae and vascular plants. If all the electrons for photosynthesis in these bacteria came from sulfide, the sulfide consumed would be one-third the amount emitted from the sediment.

VITA

NAME OF AUTHOR: David John Minter

PLACE OF BIRTH: Oakland, California

DATE OF BIRTH: 5 July 1954

UNDERGRADUATE AND GRADUATE SCHOOLS ATTENDED:

University of Washington
University of Oregon

DEGREES AWARDED:

Bachelor of Science, 1977, University of Washington

AREAS OF SPECIAL INTEREST:

Anaerobic bacteria
Geochemical transformations by marine bacteria
Population biology

PROFESSIONAL EXPERIENCE:

Teaching Assistant, Department of Biology, University of Oregon, Eugene, 1977-1982

AWARDS AND HONORS:

Morgenroth Scholarship, Department of Biology, University of Oregon, Eugene, 1980-1981

National Science Foundation Predoctoral Research Grant, 1981-1982.

PUBLICATIONS:

Minter, D. J. 1981. Intertidal sulfur cycle (Abstract). Estuaries 4: 247.

ACKNOWLEDGEMENTS

To those for whom I have not taken time to thank in proportion to their contributions, I now express my gratitude for worthwhile assistance and encouragement. It was James Murray of the University of Washington who introduced me to the world of anaerobes, in our discussions of the Black Sea. My advisor, Paul P. Rudy, allowed me to pursue it in the tideflats of Coos Bay. I was guided by the advice of David Bella and Ken Williamson of Oregon State University; they had earlier established a framework for understanding the dynamics of sulfur flow in estuarine sediment. Funds for this work were provided by a gift from the Department of Biology, and National Science Foundation Grant #OCE8618996 for the Improvement of Doctoral Dissertation Research.

To my family, friends, and committee members I owe special thanks for advice and encouragement, academic and otherwise. Because of their forbearance, I've had the opportunity to learn more about the sea. My understanding of it has changed, much as the body of Alonso:

"Full fathom five thy father lies;
Of his bones are coral made;
Those are pearls that were his eyes;
Nothing of him that doth fade,
But doth suffer a sea change
Into something rich and strange."

The Tempest (i, 2)

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

CHAPTER ONE

INTRODUCTION

Evolutionary Significance of the Sulfur Cycle

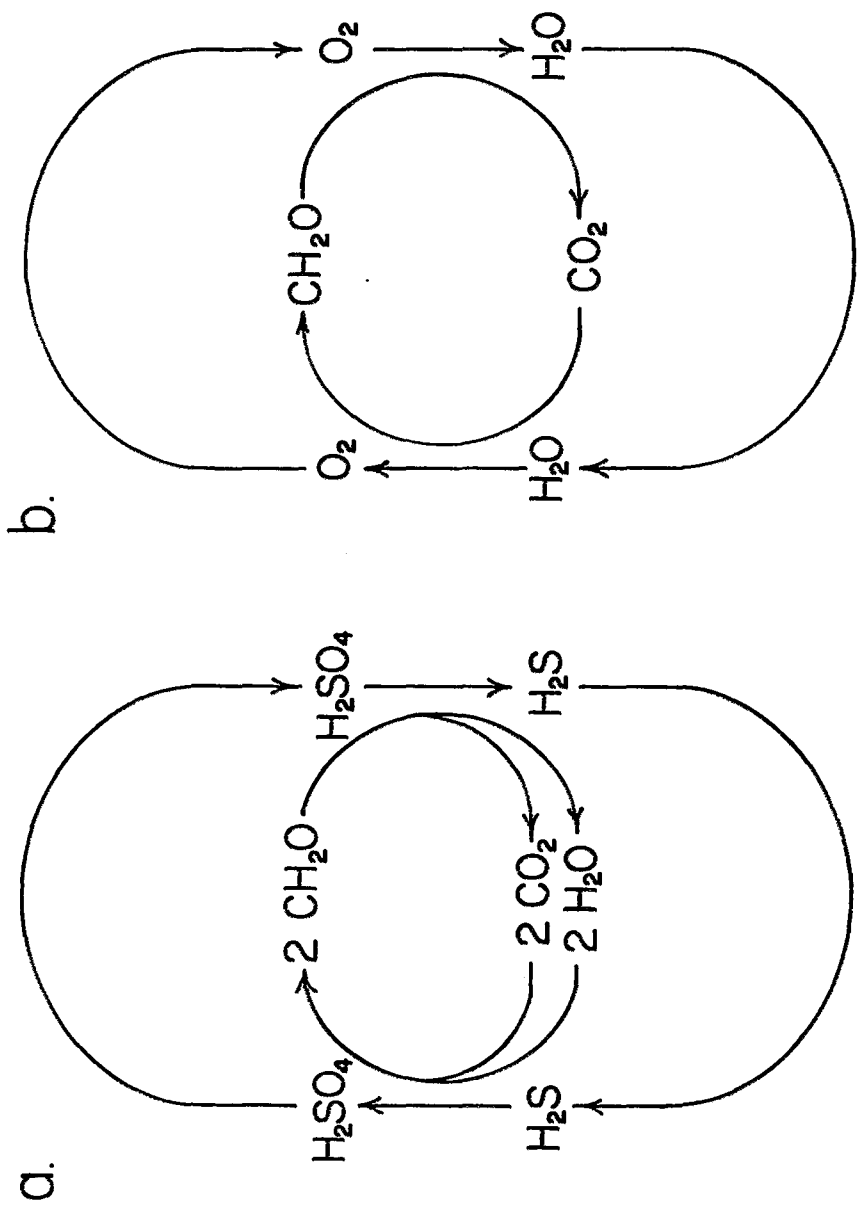
During the first billion years of life on earth, the atmosphere had little or no free oxygen. Bacteria evolved and thrived under these conditions; the earliest species being fermenting bacteria and sulfur cycling bacteria (Margulis, 1981). The fermenters derived energy from the oxidation of organic matter. External electron acceptors, if any, for this metabolism were other organic molecules. The sulfur cycling bacteria, on the other hand, were probably the first bacterial assemblage that cycled the electrons used in energy metabolism (Peck, 1974; Dickerson, 1980). Some species oxidized organized matter using sulfur compounds as electron acceptors, the other species reclaimed those electrons in order to create organic matter.

This cyclic, rather than dead-end, use of electrons is diagrammed in Figure 1.1. Photosynthetic bacteria reduce carbon atoms using electrons from sulfur compounds such as sulfide (H_2S). Sulfur compounds of higher oxidation state, such as sulfate (H_2SO_4), and organic matter are formed. Nonphotosynthetic bacteria break down this organic matter by means of respiration, using compounds such as sulfate as electron acceptors.

Figure 1.1 -- Cyclic flow of electrons in energy metabolism.

a. Sulfur photosynthesis and respiration.

b. Oxygen photosynthesis and respiration.


b.
O2
H2O
CH2O
CO2
O2
H2O
a.
H2SO4
H2S
2 CH2O
2 CO2
2 H2O
H2SO4
H2S

This general scheme of electron transfer is elaborated in a modern community of sulfur cycling bacteria. But few, if any, of these bacteria are identical with their pre-Cambrian ancestors (Peck, 1974; Postgate, 1979). Some have specialized in various aspects of the cycle, others have added accessory types of energy metabolism. There are even sulfur cycle bacteria which derive energy from reacting sulfide with molecular oxygen, a gas not present in the astmosphere until late in the evolution of prokaryotes. Nevertheless, energy metabolism in all these bacteria is based on pathways that evolved during Earth's anaerobic past. The behavior of modern species often reflect this ancestry. Two examples described in this work are: 1) the inability of oxygen-intolerant green sulfur bacteria (family Chlorobiaceae) to thrive under otherwise favorable conditions at the surface of intertidal sediment, and 2) chlorophyll synthesis in the dark by purple bacteria (families Chromatiaceae and Rhodospirillaceae) within intertidal sediment.

About two and one-half billion years ago, a new line of photosynthesizers arose. These bacteria used a more abundant source of electrons, water, in place of sulfide. Extracting electrons from water requires additional light energy (whereas extracting electrons from sulfide requires no input of energy), yet these bacteria became dominant in most environments. Not only could they find electrons in most environments, but also they liberated oxygen atoms from water in the form of molecular oxygen which was toxic to their competitors the sulfur cycle bacteria.

A new cycle was formed by the oxygen-producing photosynthesizers and some of the bacteria which consequently evolved the ability to respire with oxygen. Electrons were cycled in oxygen-containing compounds (Figure 1.1). This is identical in strategy to the sulfur cycle: electrons for photosynthesis are supplied by water, the resulting oxygen is used by respirers thus recreating water.

The abundance of molecular oxygen in the atmosphere forced sulfur cycle bacteria to retreat to habitats that remained anoxic, and to depend on oxygen cycle photosynthesizers which came to provide the bulk of organic matter for heterotrophs on Earth. The descendants of these sulfur cycle bacteria can be found in habitats of varying degrees of anoxia: eutrophic and salt lakes, hot sulfur springs on land and at mid-oceanic tectonic spreading centers, and marine sediments. Salt-water fishermen of South America even have a name for the mats of sulfide-consuming bacteria which clog their nets as they trawl the bottom: estopa, "dirty wool" (Gallard, 1977).

The microfauna and non-sulfur cycle bacteria in these low-oxygen, high-sulfide habitats have adapted in various ways to the presence of toxic sulfide (Fenchel, 1969; Fenchel and Riedl, 1970). The macroscopic community, whose ecological relationships have been studied in greater detail, also has members who have adapted. Plants (Howes, Howarth, Teal, and Valiela, 1981; Mendelssohn, McKee, and Patrick, 1981) and animals (Boaden, 1975; Reise and Ax, 1979) have been examined for evolutionary adaptation to sulfide environments. Some of these larger organisms are dependent on sulfide in the sense

that they have physiological defenses against sulfide that their potential competitors don't (Felbeck, Childress, and Somero, 1981; Powell, Crenshaw, and Rieger, 1979). Other organisms, such as the vestimentiferan worm described by Cavanaugh and others (1981), are indirectly dependent on sulfide for nutrition.

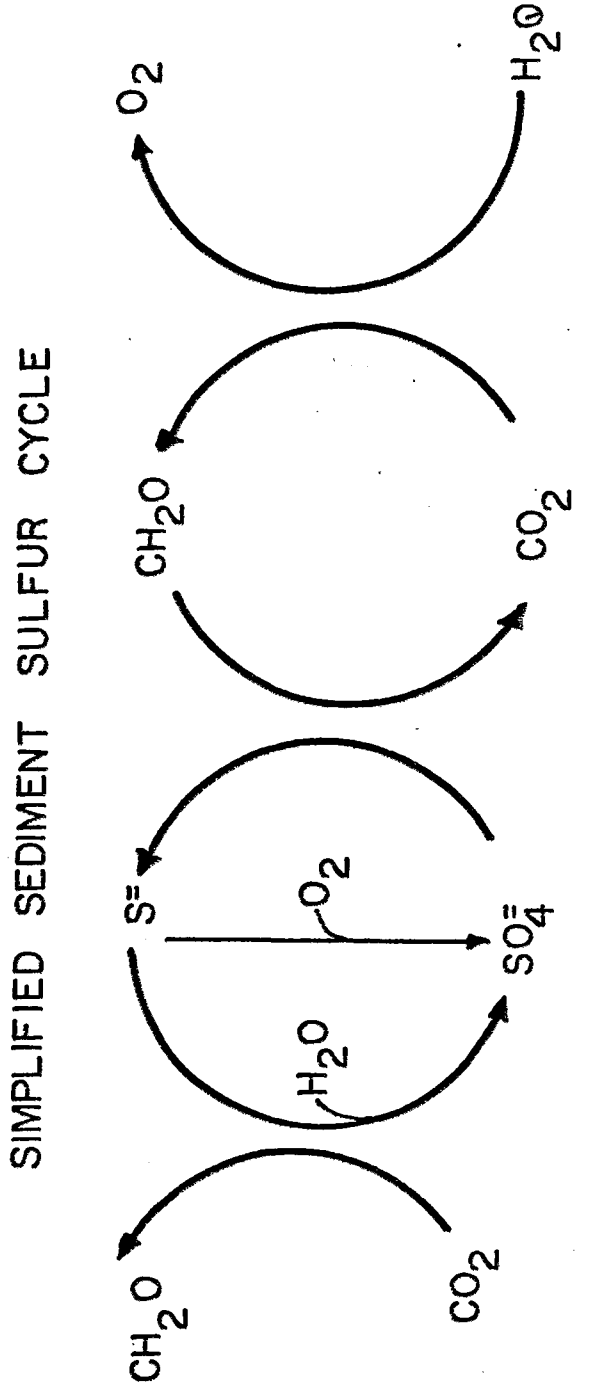
The Intertidal Sulfur Cycle

Intertidal mud is an ideal location to study the sulfur cycle bacteria, for many types of sulfur metabolism are found here. Also present are a variety of higher organisms which have adapted to sulfide.

Sulfur cycle bacteria are particularly active in estuarine sediment. Figure 1.2 shows why this is so. There is: 1) a high input of organic matter from oxygen photosynthesizers, 2) a lack of molecular oxygen which would permit oxygen respiration, 3) an abundance of sulfate from seawater, where it is about 30 millimolar, which does permit sulfur respiration, and 4) light which powers sulfur photosynthesis. Not shown in Figure 1.2 is iron which is present within the sediment and which acts reversibly to bind sulfide. In doing so it keeps the concentration of free sulfide lower than it would otherwise be, and it creates a sulfide reservoir when production exceeds consumption.

Three broad categories of bacterial metabolism are represented in Figure 1.2: sulfide production powered by the breakdown of organic

Figure 1.2. -- Generalized diagram of the interaction of the sulfur and oxygen cycles in intertidal sediment. Electrons flow from the right-hand side towards the left-hand side. For each sulfur, carbon, and oxygen transformation the thermodynamically more energetic form is shown on top.


SIMPLIFIED SEDIMENT SULFUR CYCLE
O_2
H_2O
CH_2O
CO_2
$S^=$
O_2
$SO_4^=$
H_2O
CH_2O
CO_2

matter, nonphotosynthetic sulfide consumption using molecular oxygen, and photosynthetic sulfide consumption using water.

Sulfide production is the key factor in a syntergistic system of microbial transformation in the estuary. This kind of anaerobic breakdown of organic matter prevents the accumulation of organics characteristic of low sulfate environments such that in peat bogs. The initiation of sulfide production is a self-enhancing cycle: when oxygen is locally depleted, sulfate respiration commences and sulfide accumulates. Sulfide can kill nearby organisms, thus introducing more organic matter. This increases sulfide production, then sulfide oxidation depletes oxygen in adjacent areas, and so on. In estuaries like Coos Bay this general drift towards sulfide-rich sediment can be limited by low organic input, but also it is periodically set back by catastropic oxidation by winter storms or human dredging. It may also be enhanced by diking or increased nutrient input by humans (Bella, 1975). Superimposed on large-scale catastrophic set backs and enhancement is the small-scale annual periodicity in sulfide caused by changes in bacterial activity; this small scale periodicity is what is measured in this work.

Sulfide can escape the sediment sulfur cycle by three non-biological pathways. It can bind permanently to iron in the sediment (Berner, 1970; Howarth, 1979), it can leach from the sediment at low tide as thiosulfate or other compounds (Howarth and Teal, 1980), or it can escape to the atmosphere (Adams et al., 1981; Friend, 1973; Hansen and van Gemerdan, 1972; Kellog, 1972). Atmospheric input from

marine sources is a significant part of global sulfur transport. The amount of reduced sulfur reaching the atmosphere each year from biological marine sources, including mudflats, is about 10^8 metric tons. This is as great as the physical input from oceans to the atmosphere of sulfate in the form of spray, and as great as human atmospheric input from coal-burning power plants and industrial sources.

Whatever the fate of an individual reduced sulfur molecule, it represents energy from biological sources stored as a compound not accounted for in traditional ecological studies. In the estuarine environment, this discrepancy is significant (Howarth and Teal, 1980; Peterson, Howarth, Lipshulz, and Ashendorf, 1980).

Purpose of This Work

Sulfide producers have been studied in environments unlike the muddy estuaries of the Oregon coast: Scandinavian fjords (Fenchel, 1969; Jorgensen, 1977a; Sorensen, 1979), the Black Sea (Deuser, 1970), and salt marshes of eastern North America (Howarth and Teal, 1979 and 1980; Peterson Howarth, Lipshulz and Ashendorf, 1980). Photosynthetic sulfide consumers (Fenchel and Straarup, 1971) and nonphotosynthetic sulfide consumers (Jorgensen, 1977b) have also been measured in estuarine environments unlike ours. In these settings, activities of sulfide oxidizers have never been measured directly.

The purpose of this work is to describe the natural history of sulfur cycle bacteria in Coos Bay intertidal sediment. Qualitative

and quantitative changes in activity of sulfur cycle bacteria across the bay and over the year are discussed in terms of annual periodicity in measured sulfur and carbon fluxes. Storage of biological energy forfeited by certain anaerobic bacteria in reduced sulfur compounds and the potential reclamation of that energy by other species are the quantities of particular interest.

Measurements of reclamation of energy from sulfur compounds are here limited to the upper few millimeters of sediment. This energy returned in the form of organic matter is more likely to enter food chains of higher organisms than that reclaimed deep in the sediment and includes some energy added by bacteria photosynthesis.

These are the rates that are measured: 1) photosynthetic production of organic matter in intertidal areas, 2) sulfide production by bacteria consuming organic matter in intertidal sediment, 3) carbon dioxide fixation at the sediment surface by sulfide-consuming bacteria, and 4) loss of sulfide to the atmosphere.

CHAPTER TWO

CHARACTERIZATION OF SULFUR CYCLE BACTERIA

Estaurine sulfur cycle bacteria include: 1) sulfur reducers which power the cycle by creating reduced sulfur compounds using energy released in the breakdown of organic matter, 2) nonphotosynthetic sulfur oxidizers which used reduced sulfur compounds as a source of energy, and 3) photosynthetic sulfur oxidizers which use reduced sulfur as electron sources. Estuarine sulfur reducers described by other workers include the genera Desulfovibrio (Postgate, 1979), Desulfuromonas (Phennig and Biebl, 1981), and Desulfotomaculum Widdel and Pfennig, 1977). Nonphotosynthetic sulfur oxidizers include Beggiatoa (Jorgensen, 1977b), and Thiobacillus (Adair and Gundersen, 1969; Matheron and Baulaigue, 1972). Photosynthetic sulfur oxidizers belonging to the green and purple bacteria include Chlorobium (Trüper, 1970; Matheron and Baulaigue, 1972), Prostecochloris (Trüper, 1970; Pfenning and Biebl, 1981), Chromatium (Trüper, 1970; Matheron and Baulaigue, 1972), Thiocysitis (Trüper, 1970), Thiocapsa (Trüper, 1970), Ectothiorhodospira (Trüper, 1970; Matheron and Baulaigue, 1972) and Rhodopseudomonas (Hansen and Veldkamp, 1973). Estuarine photosynthetic sulfide consumers may also include cyanbacteria, which can often perform sulfur photosynthesis in addition to oxygenic photosynthesis (Castenholz, 1977; Cohen, Padan, and Shilo, 1975; Jorgensen, Kuenen,

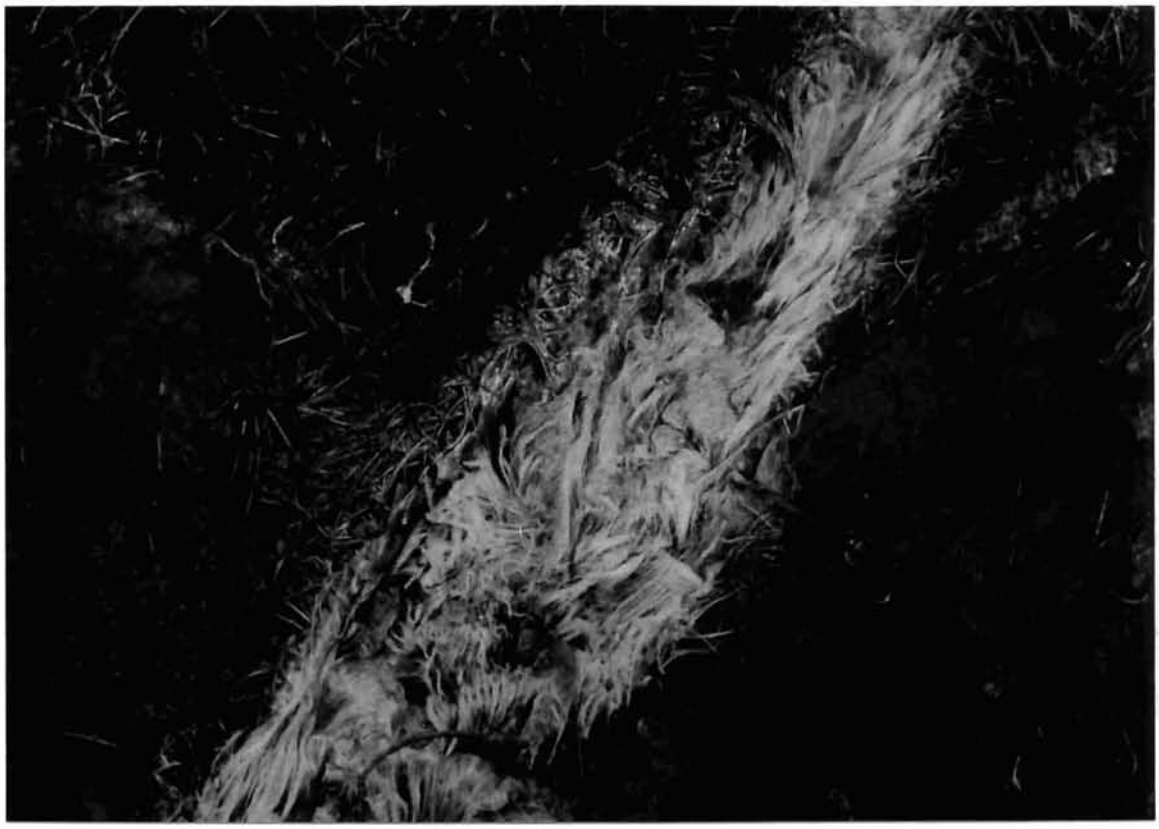
and Cohen, 1979). Oscillatoria, a cyanobacterium often found in high sulfide environments, is found in estuaries (Van Baalen, 1961).

In Coos Bay, some of these bacteria form films that can be seen in tidepools and tidal streams. On certain protected mudflats, in spoil island ponds, and in some intertidal salt marshes, photosynthetic and nonphotosynthetic sulfur oxidizers can be seen where the black sulfide layer approaches the sediment surface. Water seeping from the sediment at such locations at low tide often supports growth of Beggiatoa-like sulfur oxidizers, shown in Figures 2.1 and 2.2. In pools into which these streams flow, organic matter is covered with sulfur oxidizers as well. Figure 2.3 shows films of purple bacteria covering algae and twigs in such a pool. Figure 2.4 shows zonation of organisms at the edge of a tidepool. From right to left, one sees: 1) a brown layer of diatoms and organic matter, 2) a black band of sulfide producers with scattered blue patches of photosynthetic purple bacteria, and 3) a white band of nonphotosynthetic sulfide oxidizing bacteria.

On most Coos Bay mudflats, bacteria are not abundant enough to form visible bands or films, but they can be detected by incubating sediment in selective media. All muddy locations yielded the same types of sulfur cycle bacteria, but it was always easier to grow them from mud having higher organic content such as that at the protected Isthmus Slough location (sampling locations are shown in Figure 3.1). The South Slough location is more typical of Coos Bay intertidal sediment in that sulfur reducers were abundant but sulfur oxidizers were not.

Figure 2.1 -- Nonphotosynthetic sulfur oxidizing bacteria in a tidal stream.

Figure 2.2. -- Microscopic view of nonphotosynthetic sulfur oxidizing bacteria in a tidal stream.



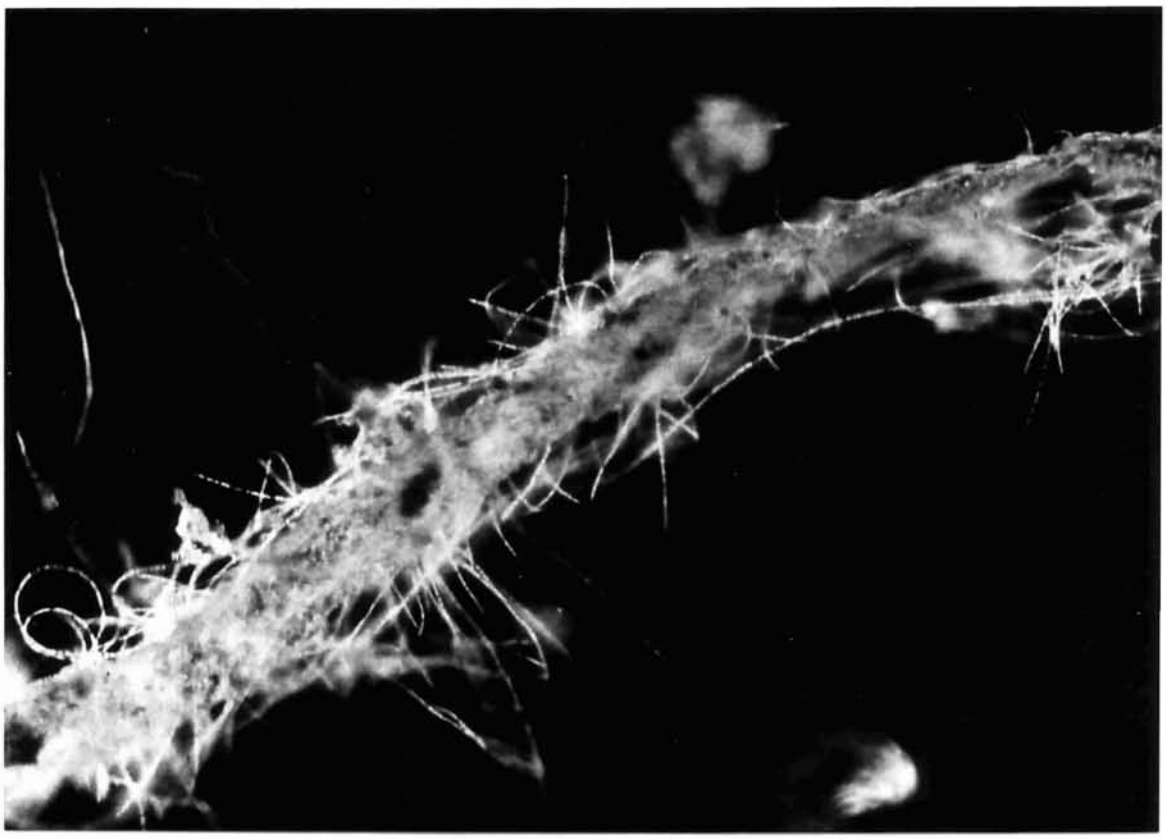


Figure 2.3. -- Photosynthetic sulfur oxidizing bacteria in a tidepool.

Figure 2.4. -- Zonation of sulfur cycle bacteria in a tidepool.

Sulfate reducers and cyanobacteria were the bacteria easiest to grow in selective media inoculated with sediment (using Medium e of Postage, 1969 and Modified Chu-11 of Waterbury and Stanier, 1981 respectively). Cells resembling Rhodopseudomonas and Chromatium, and and Thiobacillus could be grown (in Medium 2 of Pfenning and Trüper, 1981 and the medium described by Adair and Gundersen, 1969) but more attempts were needed for success. Cells resembling Chlorobium were the only green bacteria detected (using Medium 2 of Pfenning and Trüper), and it took many samples of sediment to generate a single growing culture.

Detection of green bacteria using the chromatography technique devised by Madigan and Brock (1976) for separating bacteriochlorophyll of green bacteria from chlorophyll of oxygen photosynthesizers was attempted. Samples from all locations, including wave-swept beaches where green bacteria cannot grow, showed a band on the chromatograph in the location expected for bacteriochlorophyll of green bacteria. However, the light absorbance in alcohol of material in this band did not resemble that of bacteriochlorophyll but resembled that reported for acid degraded carotenoid pigments (Riemann, 1978). Amounts of green bacteriochlorophyll, if any, were not great enough to be seen above the absorption of these unknown substances.

It is not surprising that green bacteria are sparse in Coos Bay sediment. They must have light to grow (Keister, 1978) and cannot abide oxygen, but unlike the purple bacteria, have no mechanism for

motility and cannot migrate to deeper oxygen-free layers when necessary. Even in fjord sediment covered by a shallow layer of oxygen-free water, green bacteria have not been proven active. A study by Blackburn, Kleiber, and Fenchel (1975) indicates that sulfide concentrations in the water drops faster when the sediment is illuminated with wavelengths needed by green bacteria than when illuminated by those needed by purple bacteria. However, this sulfide loss could instead be due to chemical oxidation by the oxygen produced by diatoms and cyanobacteria using the same wavelengths.

CHAPTER THREE

DISTRIBUTION OF PURPLE BACTERIA

All muddy sample locations in this study of Coos Bay contained bacterial sulfur reducers, nonphotosynthetic sulfur oxidizers, and photosynthetic sulfur oxidizers that could be cultured in selective media. The ability to estimate sulfur cycle activity by measuring abundance of these bacteria would be a quick and simple method of surveying the estuary, but in fact abundances of different sulfur cycle bacteria show varying dependence on the rate of sulfur flux.

Limited sampling of sulfur reducers done for this study show that they are most abundant several centimeters beneath the sediment surface, a distribution described for other intertidal locations (Abdollahi and Nedwell, 1970; Jorgensen, 1977a). Although in these studies maximum numbers occurred at approximately the same depth in the sediment as the maximum total sulfide production over the year, these numbers do not vary predictably over time and hence are poor indicators of activity change from month to month. The ability of these bacterial populations to maintain viable cells in numbers independent of measured sulfide production is in part due to the ability of certain species to ferment as well as respire (Vosjan, 1975) and the ability of certain species to grow symbiotically with methane-producing bacteria (Bryant, Campbell, Reddy, and Crabill,

1977). In this symbiotic union, sulfur reducers shunt excess electrons to methanogens via hydrogen gas rather than using the electrons to reduce sulfur internally.

One filamentous nonphotosynthetic sulfide-consuming bacterium often characteristic of intertidal mud rich in sulfide, Beggiatoa, also does not vary predictably with sulfide production (Jorgensen, 1977b). Beggiatoa and other filamentous species were too sparse in the upper layers of Coos Bay sediment to be estimated accurately under a microscope. The single-celled sulfur oxidizers, known to exist from enrichment cultures made from Coos Bay sediment, were too small and nondescript to be counted directly.

I was unable to enumerate by incubation in selective media the photosynthetic sulfide-consuming bacteria in Coos Bay sediment, but the bacteriochlorophyll of purple bacteria in the sediment did seem to vary in proportion to sulfide produced (described in Chapter Four of this work). The abundance of bacteriochlorophyll was the criterion for selecting the two locations studied intensively: South Slough and Isthmus Slough. Abundance was measured at all ten locations (Figure 3.1) during 1981 to detect any episodes of increased sulfur cycle activity.

Determinations of bacteriochlorophyll of purple bacteria and the chlorophyll of cyanobacteria, diatoms, and vascular plants (when present) were made for the upper 1 mm and the upper 1.5 cm of sediment each month at the ten locations. Determinations of bacteriochlorophyll

Figure 3.1. -- Sampling locations in Coos Bay estuary. All samples collected at a height of +1 meter relative to Mean Lower Low Water.

a. Kuntz Ranch. Silty mud, north-east side of bridge where South Slough Santuary access road crosses.

b. South Slough. Mud, 100 meters south of Ferrei Point.

c. Ferrei Point. Muddy sand, promentory east of the dike protecting the old Ferrei Ranch.

d. Bluff Beach. Sand, 50 meters west of Ferrei Point.

e. Loftus Beach. Sand, north end of sandy beach adjacent to Fossil Point.

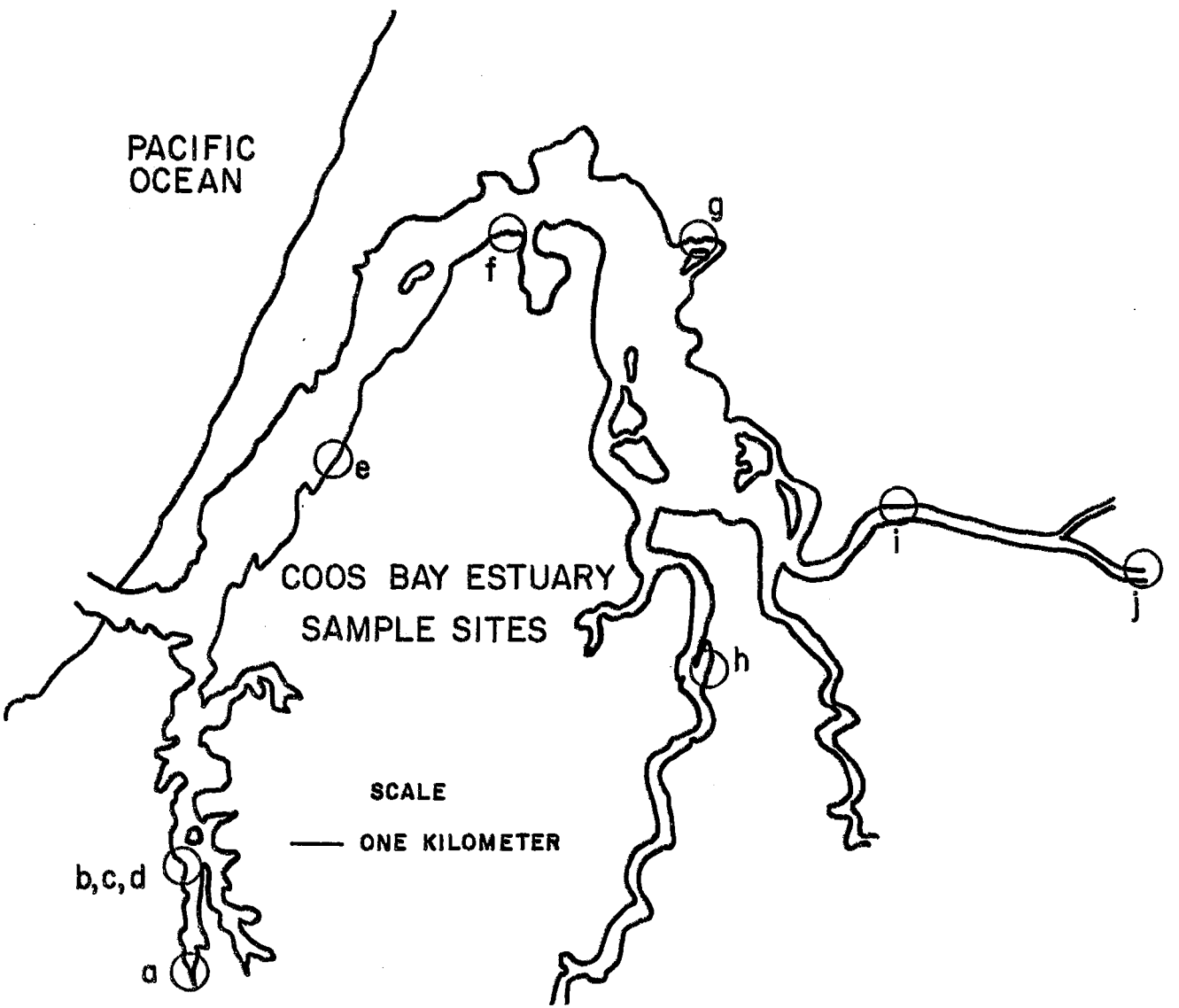
f. Airport Point. Sand, north side of public boat ramp.

g. Kentuck Inlet. Mud, one kilometer west of bridge across slough.

h. Isthmus Slough. Mud, 50 meters north of stream flowing under road on east side of diked area.

i. Coos River. Mud, north side of river one kilometer east of Allegany Bridge.

j. Myrtle Tree Park. Mud, riverbank upstream from public boat launch.


PACIFIC
OCEAN
COOS BAY ESTUARY
SAMPLE SITES
SCALE
ONE KILOMETER
a
b,c,d
e
f
g
h
i
j

were made for the upper 10 cm of sediment, in 1 cm increments, each month at the South Slough and Isthmus Slough locations.

South Slough (Figure 3.2) is a rather steep, muddy location typical of Coos Bay. Its sediment organic content is about 10% by weight. It has no measurable bacteriochlorophyll at any time of year, and no plants other than diatoms, cyanobacteria, and an occasional patch of Enteromorpha. The sediment is inhabited by large crustaceans, clams, and worms.

Isthmus Slough (Figure 3.3) is a flat location with sediment of 20% organic content by weight; it is representative of only limited parts of Coos Bay. It has measurable amounts of bacteriochlorophyll during most of the year. During the summer there is a growth of vascular plants normally found at very high intertidal locations. Samples taken at Isthmus Slough were from an area far from shore, where during the summer only sedge-family member Eleocharis parvula was present. Only very small invertebrate animals were found to be living underneath the mat of Eleocharis.

Methods

Chlorophyll of organisms mixed into sediment is more difficult to quantify than that of planktonic organisms, which can be filtered from the water in which they are suspecded. Very few techniques for measurement of chlorophyll in sediment have been published. Difficulties and methods developed in this study for dealing with these difficulties are described below.

Figure 3.2. -- South Slough sampling location at low tide, June 1981.

Figure 3.3. -- Isthmus Slough sampling location at low tide, June 1981.

Difficulties

Water

Drying of sediment alters chlorophyll to an unpredictable degree, extraction must be performed with an organic solvent miscible with water such as methanol. Extraction times are longer than for dry sediment in nonmiscible solvents, but loss due to decomposition during this longer extraction is negligible compared to experimental variation.

Adsorption

Chlorophyll adsorbs onto sediment, to different degrees according to the type of sediment. The problem is minimized by using a large volume of methanol relative to the sample, and varying the volume of methanol according to the amount of water in the sample so that the concentration of water in all extracts is the same.

Degradation

Sulfide, organic acids, and other reactive compounds are present in sediment and its pore water. Alteration of chlorophyll by such compounds during extraction must be minimized by extracting at a low temperature in the presence of a base.

Filtration

Before measurement, chlorophyll in methanol solution must be separated from sediment particles and from the salts which precipitate when salt water is added to alcohol. These particles can be removed by vacuum filtration through a membrane filter.

Analysis

In anaerobic mud, degradation of chlorophyll and its breakdown products occurs very slowly. Phaeophytin is typically found in large amounts, giving methanol extracts with a broad light absorption peak rather than the narrow peak formed by intact chlorophyll alone. The location of this peak varies slightly according to the pH and water content of the extract, so peak height must be measured directly by scanning a wide spectrum rather than measuring the absorbance at some expected wavelength.

Standard Technique Used in This Study

Collection

Approximately the upper 1 mm of sediment was scraped away with a scalpel; a sample of about 1 g was placed in a vial and immediately cooled to the temperature of solidified carbon dioxide.

The upper 1.5 cm of sediment was sampled by placing a plastic petri dish upside down into the mud, then sliding the other half (with part of the lip removed) underneath. This intact disk was secured

with a rubber band and cooled immediately to the temperature of solidified carbon dioxide.

The upper 10 cm of sediment was sampled as a core, using a long 10 cm diameter polyacrylate tube driven into the sediment with a rubber mallet. In the laboratory, this core was removed and sliced into 1 cm disks.

Storage

Samples were stored up to two weeks at -15°C, in plastic bags to prevent desiccation.

Subsampling

The 1 cm thick disks from petri dish and core samples were broken into small pieces using a screwdriver struck with a rubber mallet. These pieces were variable in size, but each had fractures perpendicular to the face thus giving equal proportions of horizontal layers. Three pieces, of about 2 g each, were selected. Each was weighed, then placed into a screwcap vial. Pure methanol, with enough NaOH solution added to make a final concentration of 10^{-4} molar, was added to the vials. For each gram of sediment, 20 ml of methanol was used for extraction.

Extraction

Samples were shaken as soon as the sediment had thawed, then

placed in the dark at 4°C. After 24 hours they were shaken again, then allowed to warm to room temperature before filtering.

Filtration

The supernatant methanol in each vial was poured into a membrane filtering apparatus. A faucet aspirator was used to draw the extract through a methanol-resistant membrane filter, thus removing any sediment or precipitated salt.

Absorbance Measurements

Light absorbance of each methanol extract was measured using a cuvette of 10 cm path length in a Cary Model 14 recording scanning spectrophotometer between 600 nm and 1000 nm wavelengths. Each extract was measured, acidified with HCl to a final concentration of 10^{-3} molar, and measured again. If too much acid is added, a broad absorption peak between 600 nm and 800 nm wavelengths will appear due to creation of carotenoid breakdown products (Riemann, 1978).

Chlorophyll Determination

Pigments were estimated using an algorithm based on the magnitude of absorbance peak heights before and after acidification (Marker, 1972). The equations used were:

$$\text{chlorophyll} = 3.0 \times (C_b - C_a) \times (V / L)$$

$$\text{phaeophytin} = (C_b - 3.0 \times (C_b - C_a)) \times 13.1 \times 1.5 \times (V / L)$$

$$\text{bacteriochlorophyll} = 1.3 \times (B_b - B_a) \times 21.6 \times (V / L)$$

$$\text{bacteriophaeophytin} = (B_b - 1.3 \times (B_b - B_a) \times 21.6 \times 4.3 \times (V / L)$$

where C_b is absorption at the peak located near 665 nm wavelength before acidification, C_a is absorption at that peak after acidification, B_b is absorption at the peak located near 770 nm wavelenth before acidification, and B_a is absorption at that peak after acidification. The factors of 3.0 and 1.3 assume a ratio of before to after acid peak heights of 1.5 and 4.3, respectively. The factor 13.1 is from the extinction coefficient of 76.1 ml / mg x cm (Talling and Driver, 1961), the factor 21.6 from 46.2 ml / mg x cm (Smith and Benitez, 1955). The volume of extract measured in ml is V, the cuvette path length in cm is L.

Correction was made for absorption by water and carotenoid breakdown product. Bacteriochlorophyll from green bacteria was assumed to be absent.

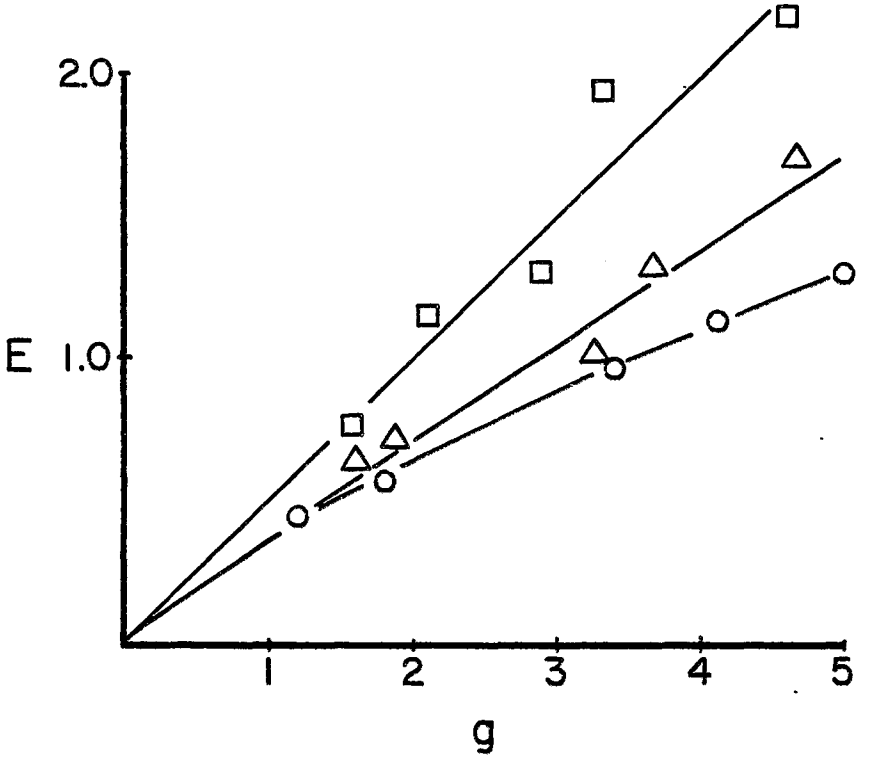
Results

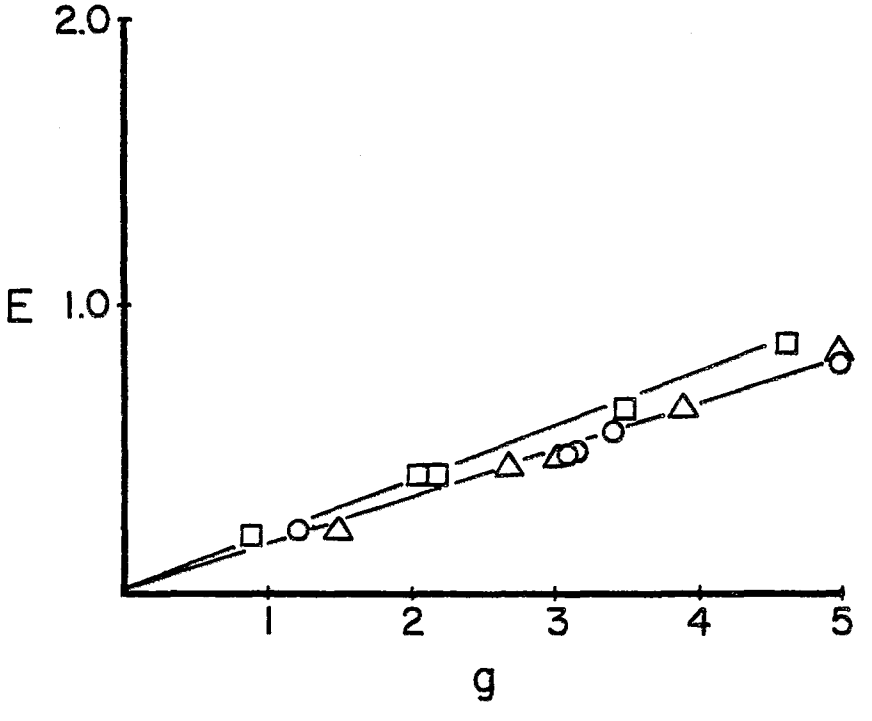
Extraction Techniques

Chlorophyll can be extracted from sediment using methanol and acetone; the results of such extractions of homogenized mud are shown in Figure 3.4. For large amounts of sediment, light absorption due to chlorophyll is linearly proportional to sample weight when extraction is performed with a constant volume of acetone, but not when performed with constant volumes of methanol. Normalization of water

Figure 3.4. -- Light absorbance at 665 nm wavelength of acetone (squares), methanol (circles), and proportional methanol (triangles) extracts, as a function of sediment sample weight.

Figure 3.5. -- Light absorbance at 665 nm wavelength of methanol extracts containing $MgCO_3$ (circles), NaOH (squares), or no additives (triangles), as a function of sediment sample weight.


2.0
E 1.0
1
2
3
4
5
g


2.0
E 1.0
1
2
3
4
5
g

content by adding a volume of methanol proportional to sample weight results in a straight line. The difference in slope between the proportional methanol volume method and the constant acetone volume method is that expected due to the difference in extinction coefficients of chlorophyll in the two solvents. Methanol was chosen for this study because it is less volatile than acetone, and because acetone must be centrifuged to remove fine sediment and precipitated salts since there is no acetone-resistant membrane filter.

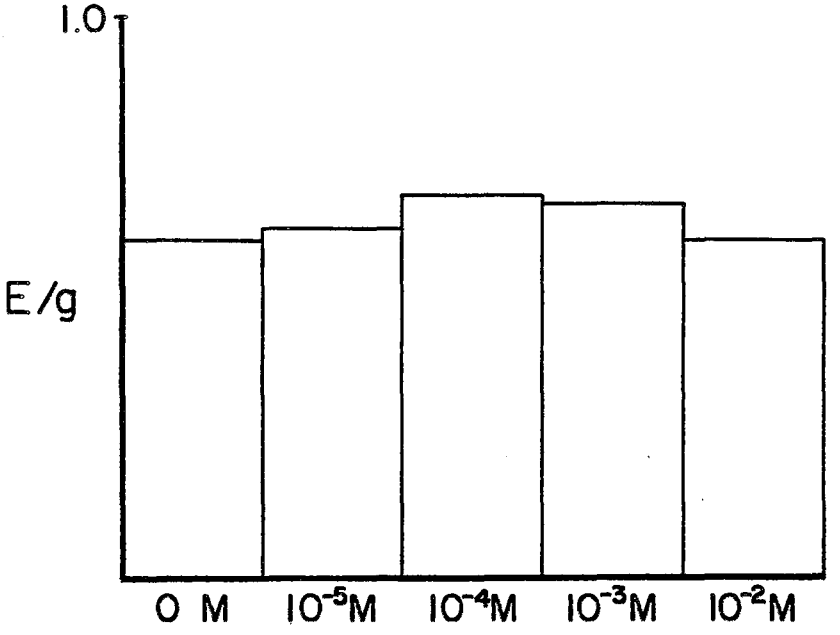
Figure 3.6 shows that addition of $MgCO_3$, a traditional anti-decomposition agent, to methanol has no effect on recovery. NaOH in 10^{-4} molar concentration does, however. The recovery is about 10% greater than with no additive. A greater or smaller concentration of NaOH gives less improvement, as shown in Figure 3.6. Estimated chlorophyll and phaeophytin concentrations based on light absorption of extracts before and after acid treatment are shown on Figure 3.7. Estimated chlorophyll is maximum, and estimated phaeophytin minimum, when NaOH is about 10^{-4} molar.

Homogenization of sediment before taking subsamples for extraction does reduce variability between subsamples, but it also reduces recovery by about 10%, as shown in Figure 3.8. Hence, determining the chlorophyll content of several replicate samples is a more accurate way of estimating the mean.

Extraction of sediment samples of various weights into methanol is shown in Figure 3.9. In this study, a standard extraction time of

Figure 3.6. -- Light absorbance at 665 nm wavelength per gram of sediment, extracted into methanol of varying NaOH concentrations.

Figure 3.7. -- Estimated amounts of chlorophyll (open) and phaeophytin (filled) per gram of sediment, extracted into methanol of varying NaOH concentrations.


1.0
E/g
0 M
10-5M
10-4M
10-3M
10-2M

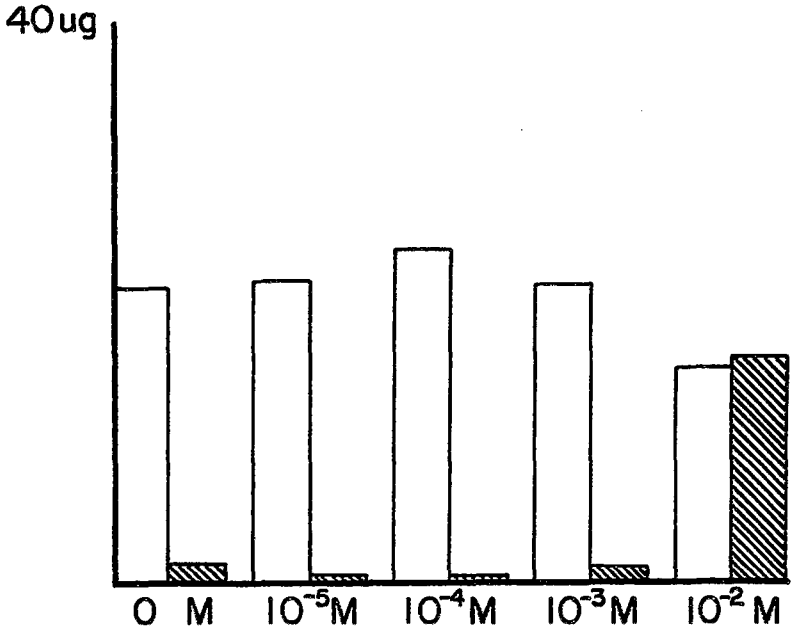
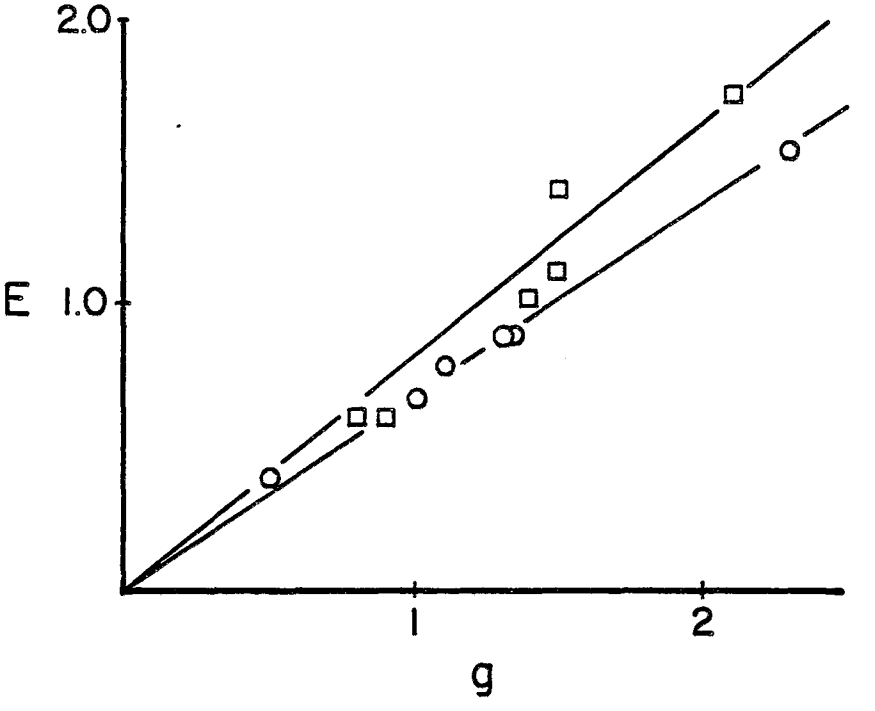
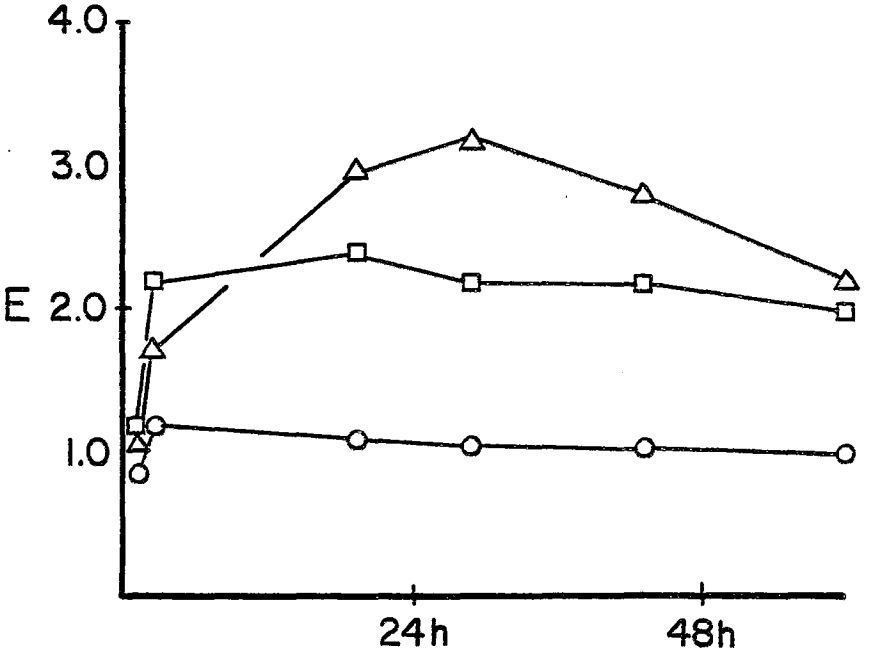

40ug
0 M
10-5M
10-4M
10-3M
10-2 M

Figure 3.8. -- Light absorbance at 665 nm wavelength per gram of sediment, for homogenized (circles) and unhomogenized (squares) samples.

Figure 3.9. -- Light absorbance at 665 nm wavelength for samples weighing 0.8 g (circles), 3.3 g (squares), and 9.7 g (triangles), as a function of extraction time.


2.0
E 1.0
1
2
g


4.0
3.0
E 2.0
1.0
24h
48h

24 hours was used since a typical sample (about 2 g) is expected to give maximum recovery in about that length of time with only slow loss thereafter.

Variation in Chlorophyll Over Small Distances

At any one location, there is greater variability between mean chlorophyll concentrations at various tidal elevations than between single concentrations at a given tidal elevation. Figures 3.10, 3.11, and 3.12 show mean chlorophyll concentrations in the upper 1.5 cm of sediment within a sampling area oriented perpendicular to the shoreline. Isopleths of chlorophyll concentration, as measured in sediment from the locations indicated by dots, are shown. Variation in concentration over different tidal elevations at South Slough, for example, is shown in Figure 3.10. The concentration is greatest in the middle of the figure, at about +1 m relative to Mean Lower Low Water, and lower both shoreward and seaward where sediment is coarser (an elevation change of about one-half meter in each direction). Phaeophytin shows the same general distribution, it is present in lower concentration. Elevation contours (not shown) roughly follow chlorophyll and phaeophytin contours; there is a sand spit immediately to the right side of each figure.

Chlorophyll concentration at Isthmus Slough has the same general trend, but due to the more gradual slope there only sediment above +1 m was sampled (Figure 3.11). Phaeophytin, unlike chlorophyll, is most concentrated near the shore and decreases seaward. High

Figure 3.10. -- Contours of chlorophyll and phaeophytin concentrations in upper 1.5 cm of sediment at South Slough in July 1981. The 4 m-wide strip sampled is perpendicular to the shoreline, its upper edge is aligned with the start of terrestrial plants (in these diagrams, at the top of the page). A sand spit is located immediately outside of the sampling area, to the right-hand side of these diagrams.

a. Chlorophyll, µg per g sediment.

b. Phaeophytin, µg per g sediment.

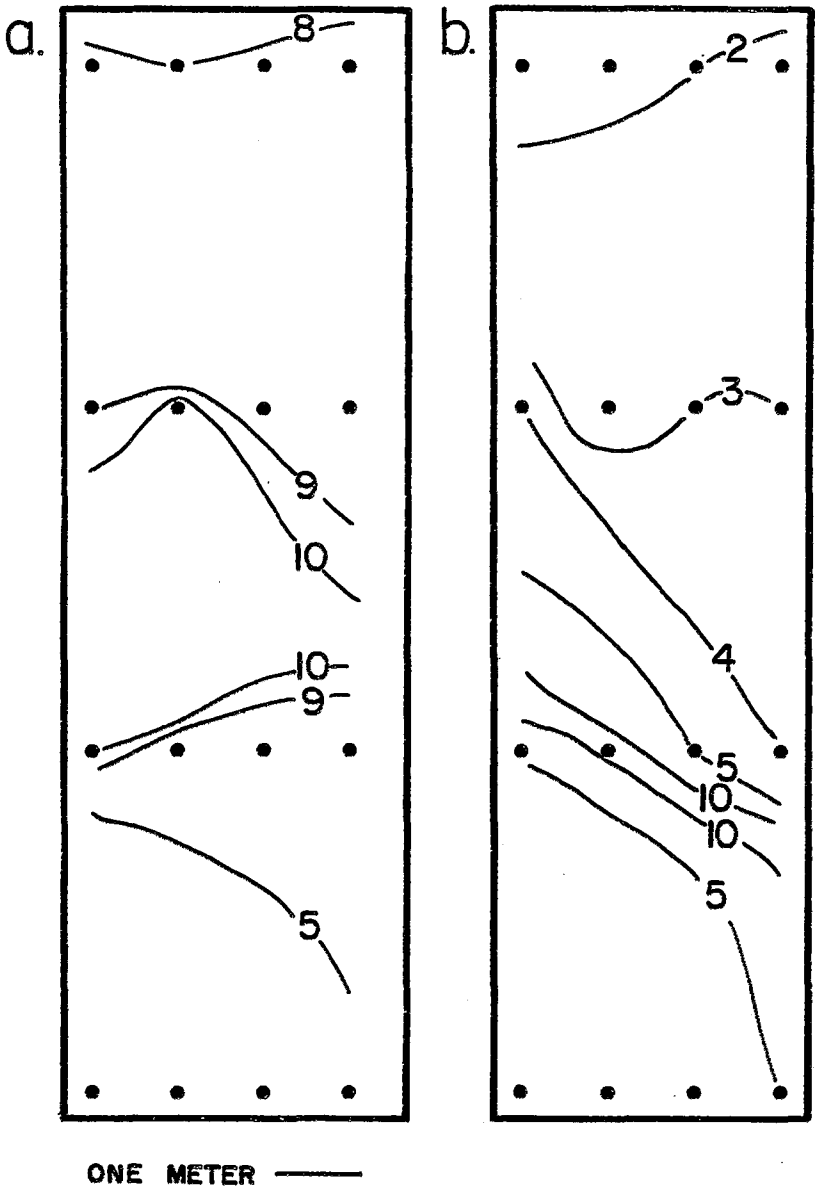

a.
8
9
10
10
9
5
b.
2
3
4
5
10
10
5
ONE METER

Figure 3.11. -- Contours of chlorophyll and phaeophytin concentrations in upper 1.5 cm of sediment at Isthmus Slough in January 1982. The 4 m-wide strip sampled is perpendicular to the shoreline, its upper edge is aligned with the start of terrestrial plants (in these diagrams, at the top of the page).

a. Chlorophyll, µg per g sediment.

b. Phaeophytin, µg per g sediment.

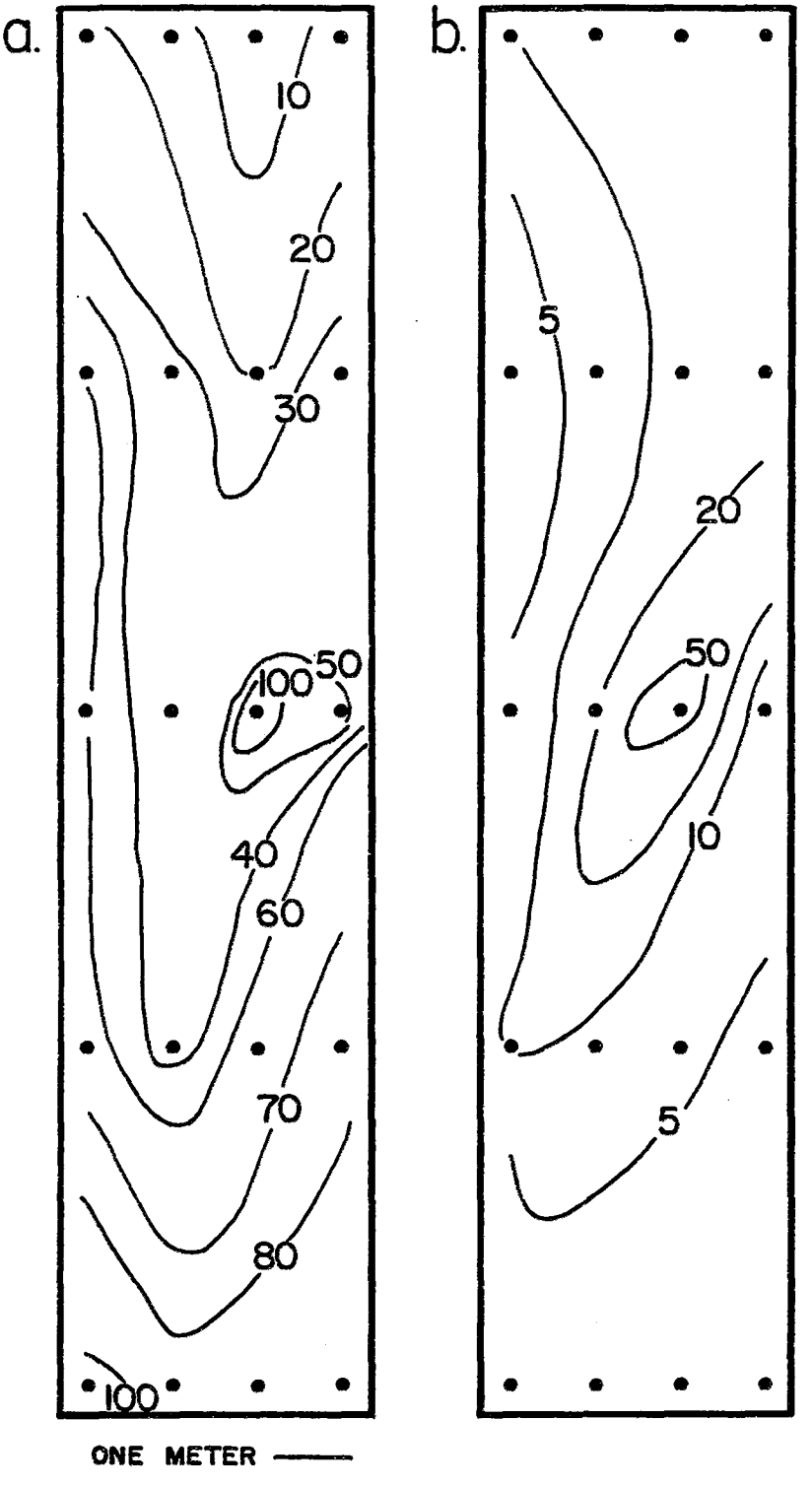
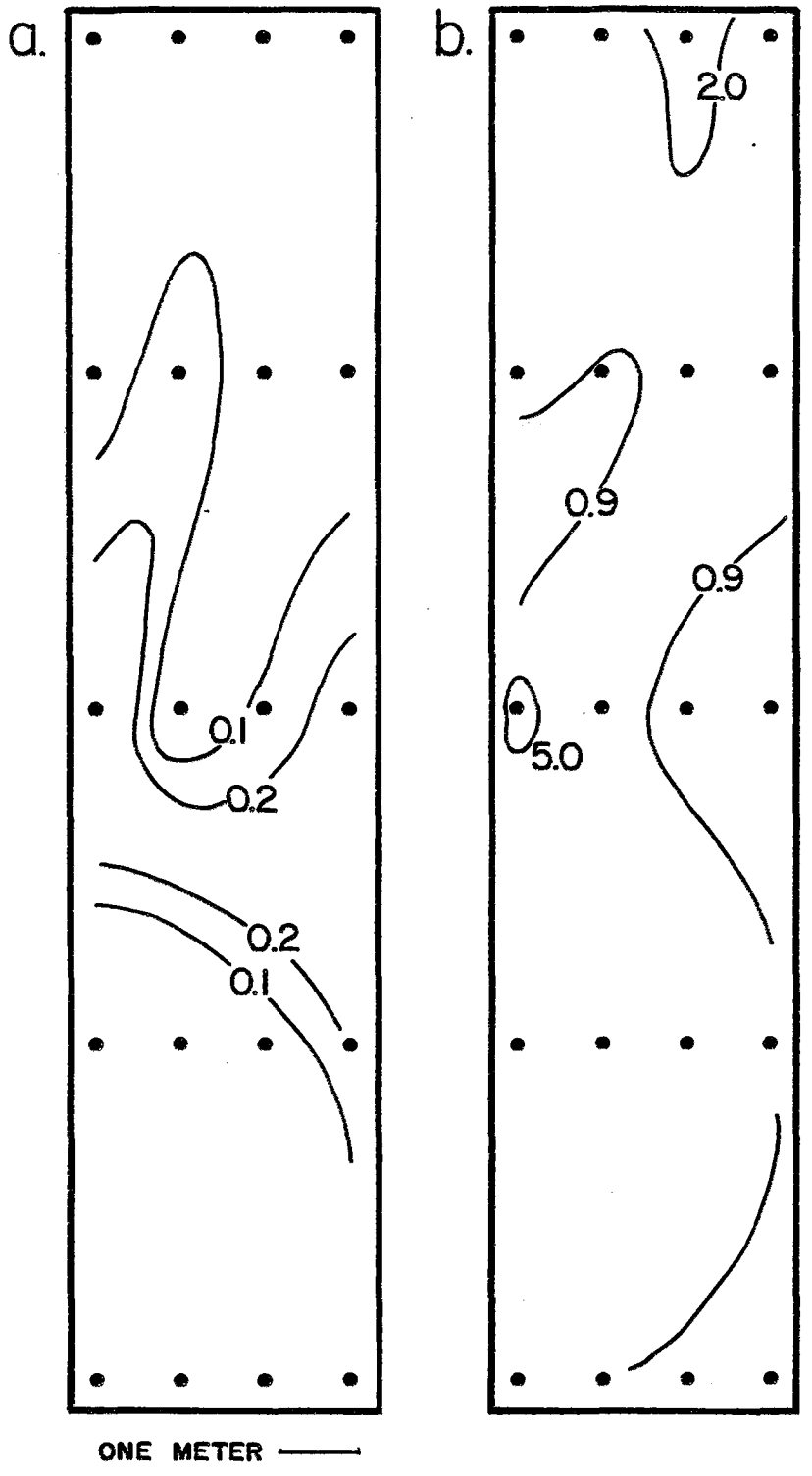

a.
10
20
30
50
100
40
60
70
80
100
b.
5
20
50
10
5
ONE METER

Figure 3.12. -- Contours of bacteriochlorophyll and bacteriophaeophytin concentrations in upper 1.5 cm of sediment at Isthmus Slough in January 1982. The 4 m-wide strip sampled is perpendicular to the shoreline, its upper edge is aligned with the start of terrestrial plants (in these diagrams, at the top of the page).

a. Bacteriochlorophyll, µg per g sediment.

b. Bacteriophaeophytin, µg per sediment.


a.
0.1
0.2
0.2
0.1
b.
2.0
0.9
0.9
5.0
ONE METER

levels of phaeophytin near shore are probably due to the greater accumulation of exogenous plant material there; this would also account for the greater concentration of phaeophytin at the protected Isthmus Slough location than the exposed South Slough location.

Bacteriochlorophyll at Isthmus Slough (Figure 3.12) also shows greatest abundance away from the shore.

Variation in Chlorophyll Over Large Distances

Chlorophyll concentration varies with sediment type. Sandy, low organic-content sediment shows less chlorophyll and phaeophytin than fine, high organic-content sediment (Figures 3.13 and 3.14). Furthermore, brackish water sediments such as that found at Coos River and Myrtle Tree Park locations show less chlorophyll and phaeophytin than similar sediments in salt water locations.

Of the ten sampling locations, only Isthmus Slough had bacteriochlorophyll of purple bacteria in measurable amounts. Bacteriochlorophyll was, however, abundant in salt marsh and spoil island sediments not included in this study.

Variation in Chlorophyll Over Time

Chlorophyll and phaeophytin concentrations showed no predictable change over the year other than a slight rise in phaeophytin during summer months at some locations (Figures 3.13 and 3.14).

Figure 3.13 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1 mm of sediment during 1981.

a. Kuntz Ranch.

b. South Slough.

c. Ferrei Point.

d. Bluff Beach.

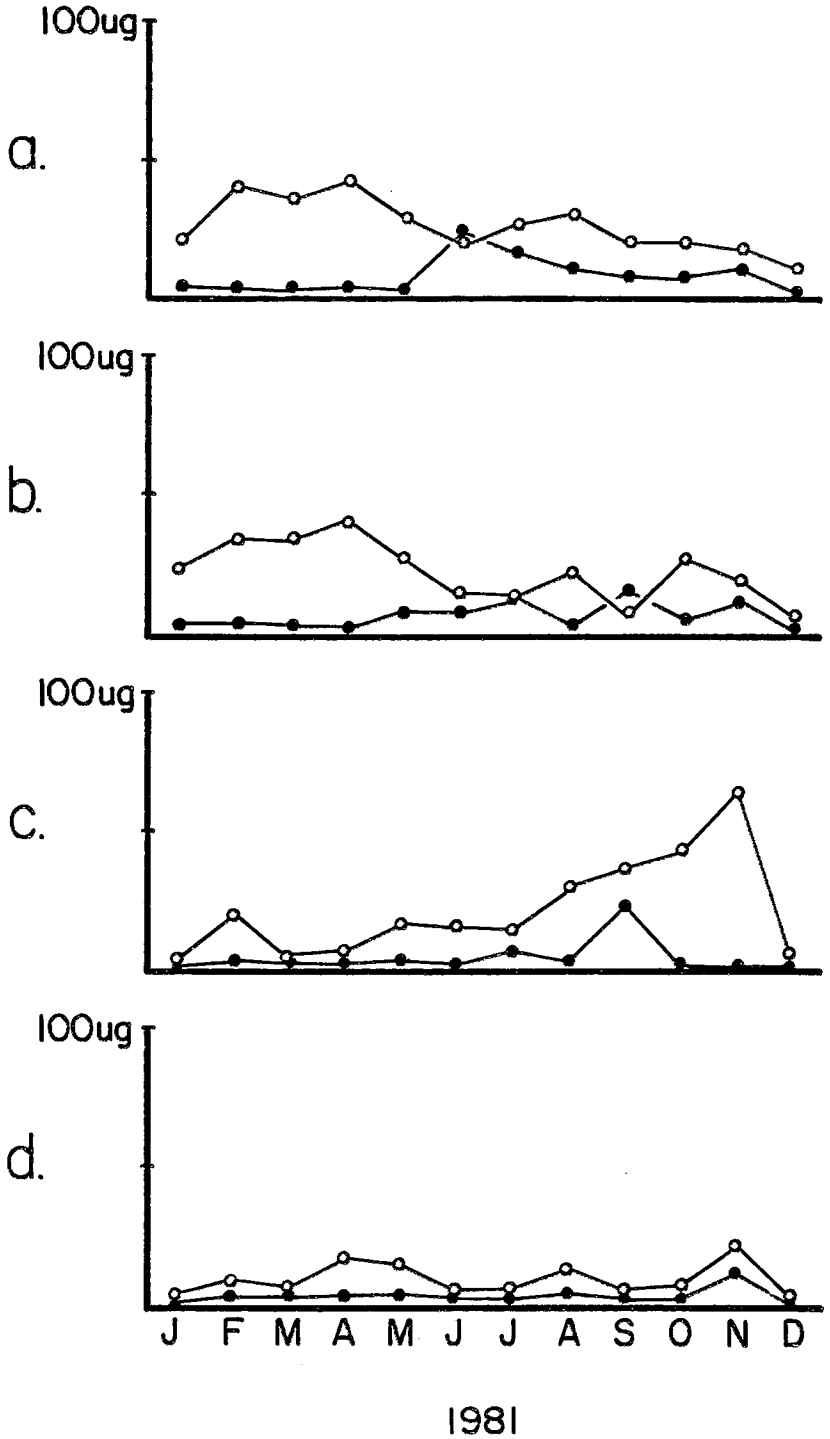

100ug
a.
100ug
b.
100ug
c.
100ug
d.
J F M A M J J A S O N D
1981

Figure 3.13 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1 mm of sediment during 1981.

e. Loftus Beach.

f. Airport Point.

g. Kentuck Inlet.

h. Isthmus Slough.

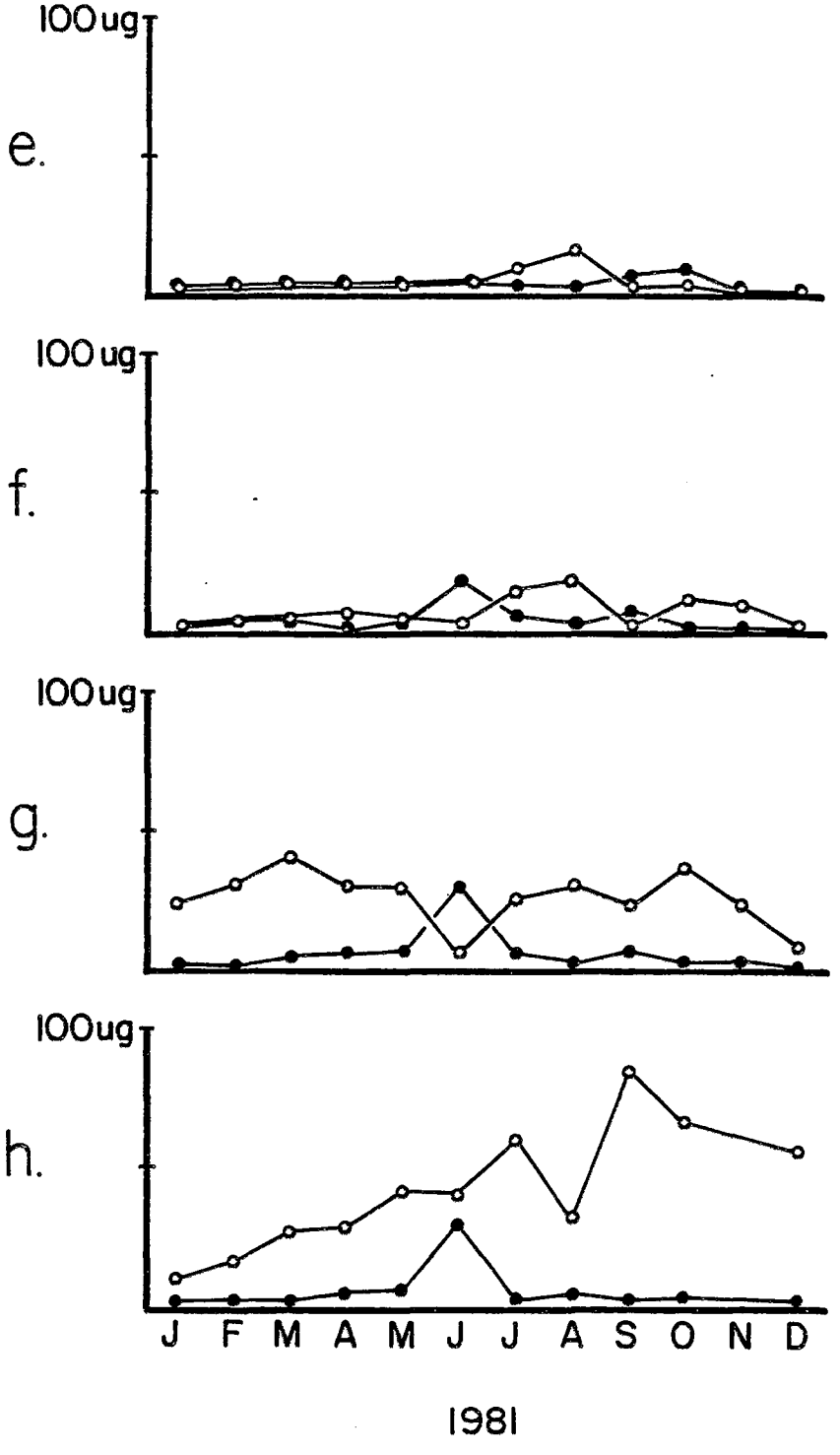

100ug
e.
100ug
f.
100ug
g.
100ug
h.
J F M A M J J A S O N D
1981

Figure 3.13 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1 mm of sediment during 1981.

i. Coos River.

j. Myrtle Tree Park.

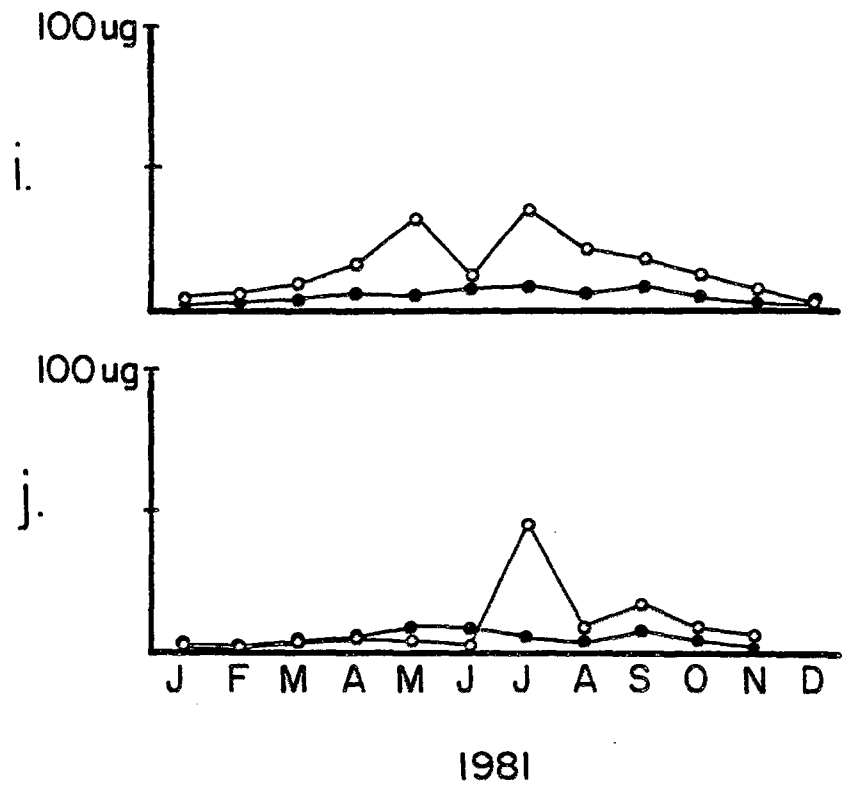

100ug
i.
100ug
j.
J F M A M J J A S O N D
1981

Figure 3.14 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1.5 cm of sediment during 1981.

a. Kuntz Ranch.

b. South Slough.

c. Ferrei Point.

d. Bluff Beach.

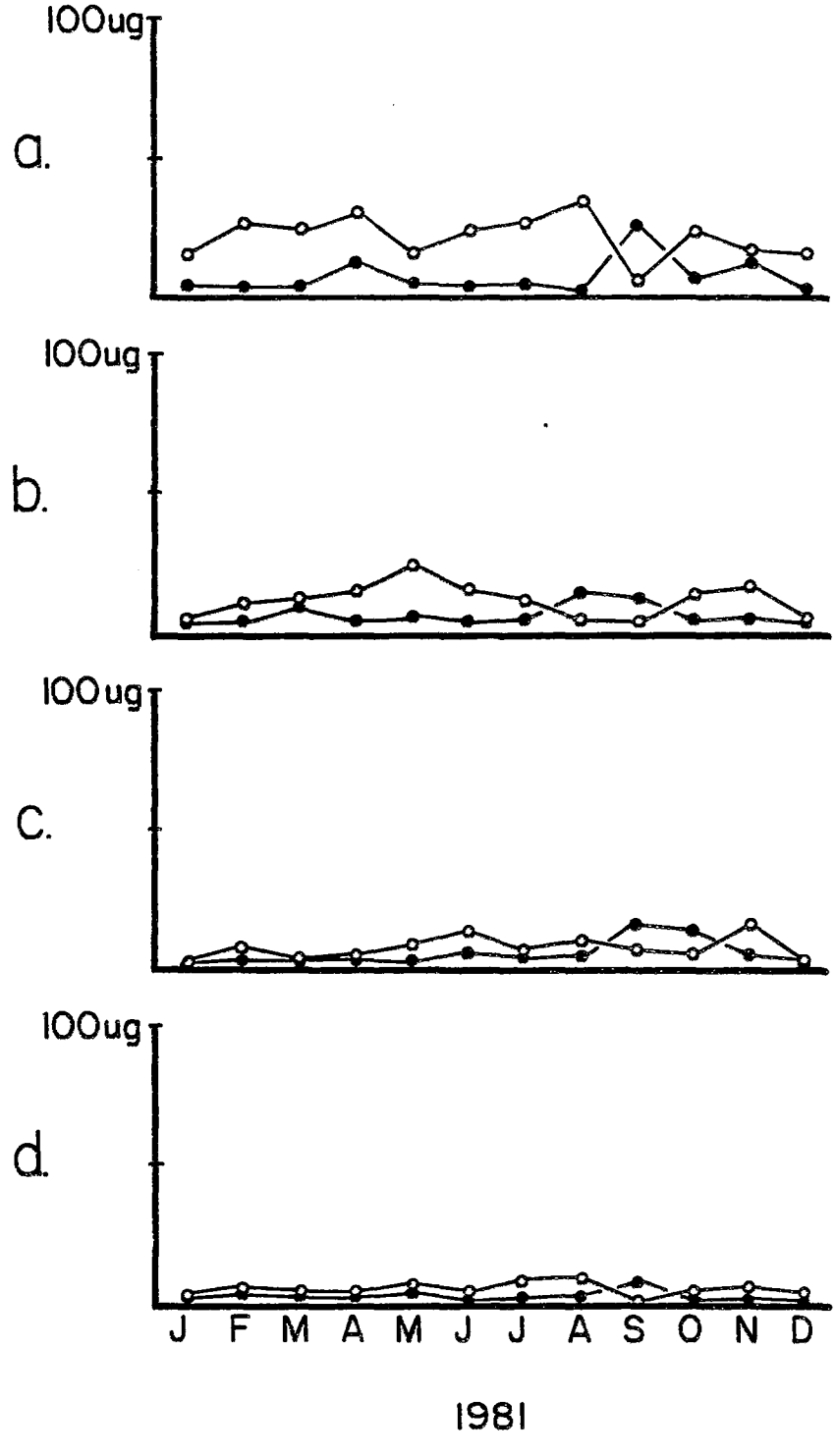

100ug
a.
100ug
b.
100ug
c.
100ug
d.
J F M A M J J A S O N D
1981

Figure 3.14 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1.5 cm of sediment during 1981.

e. Loftus Beach.

f. Airport Point.

g. Kentuck Inlet.

h. Isthmus Slough.

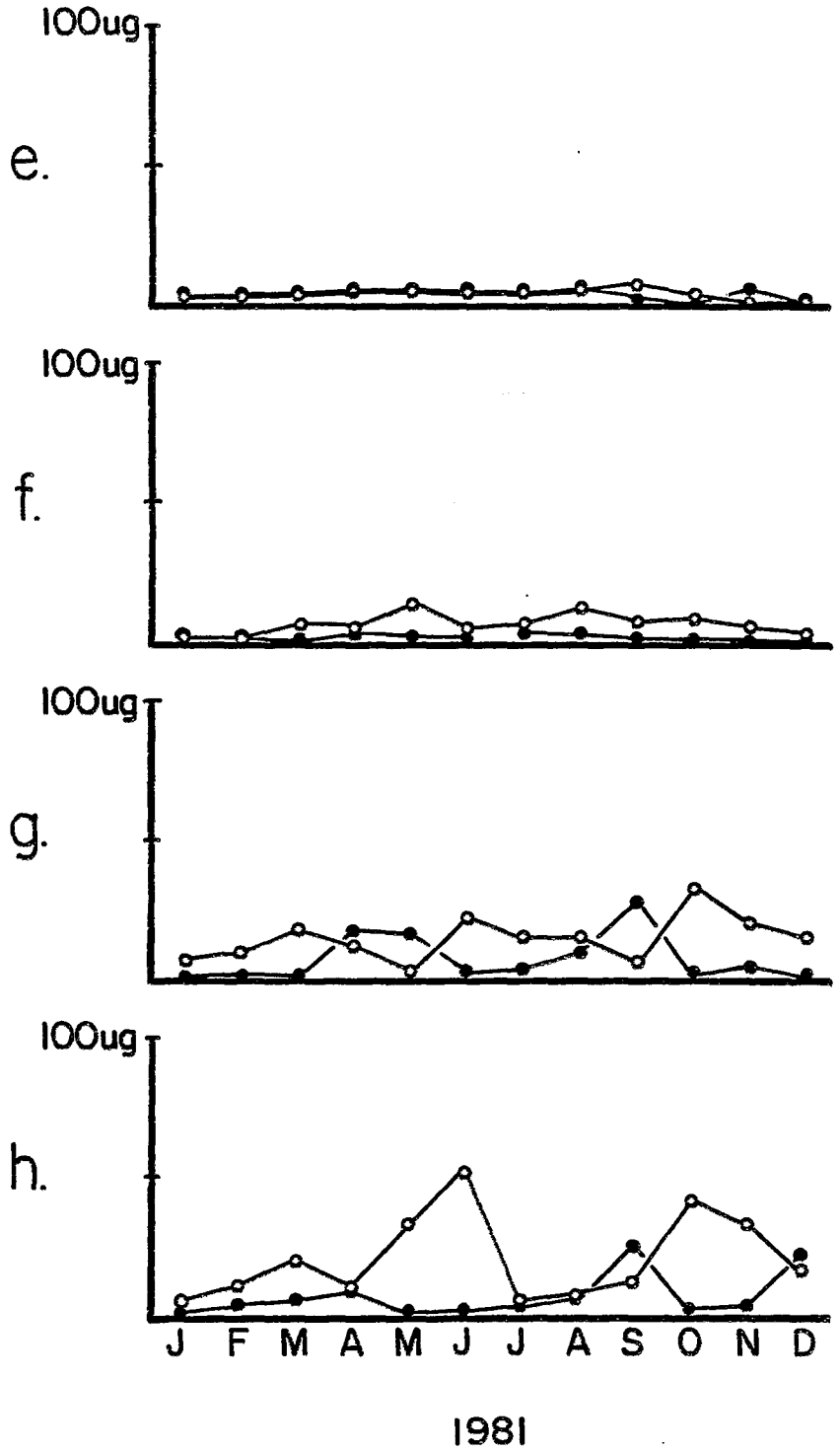

100ug
e.
100ug
f.
100ug
g.
100ug
h.
J F M A M J J A S O N D
1981

Figure 3.14 -- Concentration of chlorophyll (open circles) and phaeophytin (closed circles) in upper 1.5 cm of sediment during 1981.

i. Coos River.

j. Myrtle Tree Park.

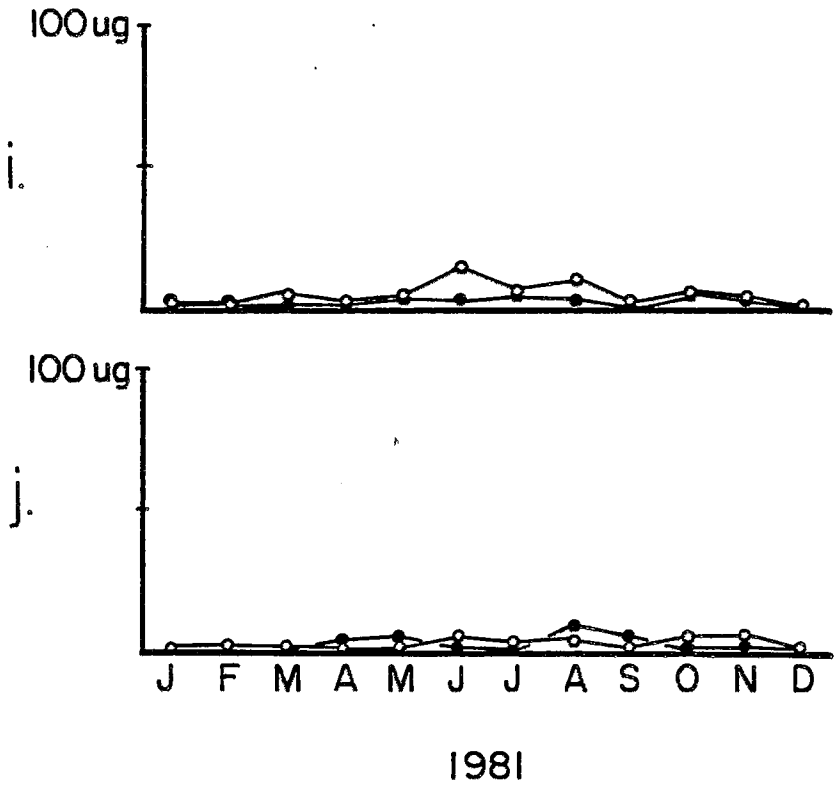

100 ug
i.
100 ug
j.
J F M A M J J A S O N D
1981

Figure 3.15 -- Concentration of bacteriochlorophyll (open circles) and bacteriophaeophytin (closed circles) in upper 1 mm of Isthmus Slough sediment during 1981.

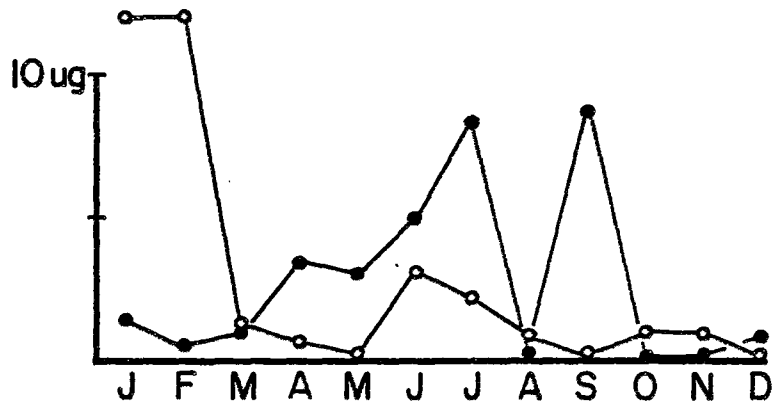
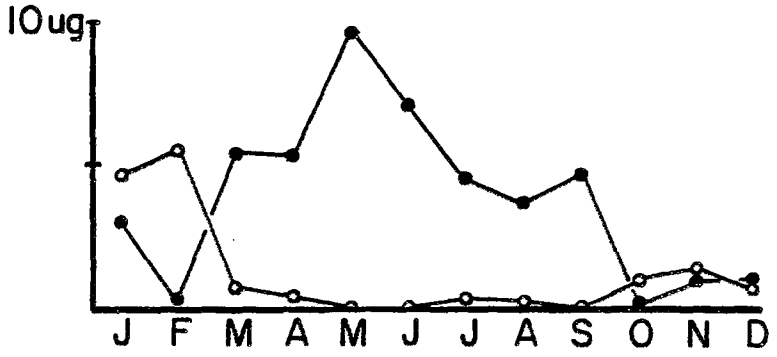

10 ug
J F M A M J J A S O N D

Figure 3.16 -- Concentration of bacteriochlorophyll (open circles) and bacteriophaeophytin (closed circles) in upper 1.5 cm of Isthmus Slough sediment during 1981.


10ug
J F M A M J J A S O N D

Variation in Chlorophyll Over Depth in Sediment

Comparison of Figure 3.13 with Figure 3.14 shows that the upper 1 mm of sediment contains more chlorophyll per gram sediment than the upper 1.5 cm.

Chlorophyll and phaeophytin are found far deeper in the sediment than the 1.5 cm routinely sampled. Cores of mud collected monthly at South Slough and Isthmus Slough (Figure 3.17) show this pigment at depth. There is no predictable trend in concentration profiles over the year, but in general there is more intact chlorophyll than phaeophytin during the winter and the reverse during the summer.

Bactiochlorophyll and Bacteriophaeophytin at Isthmus Slough show greatest concentrations at two horizons within the sediment. The first horizon, at the sediment surface, is seldom as great as the second horizon several centimeters deep. The depth of the second horizon varied between monthly samples since precisely the same spot cannot be sampled on consecutive months at the slowly healing Isthmus Slough location, but the level varied according to the depth of a wood fiber layer in the sample core. This layer, a couple of centimeters thick, lies under the sediment which has been deposited in the decade since this area was used as a log storage location. Bacteriochlorophyll concentrating at this location also differs from chlorophyll concentration in that there is a predictable trend over the year: it is high in winter, and low in summer.

Figure 3.17. -- Concentration of chlorophylls and phaeophytins as a function of depth in sediment.

a. January 1981. Shown are South Slough data for chlorophyll (1.) and phaeophytin (2.), and Isthmus Slough data for chlorophyll (3.), phaeophytin (4.), bacteriochlorophyll (5.), and bacteriophaeophytin (6.).

a.

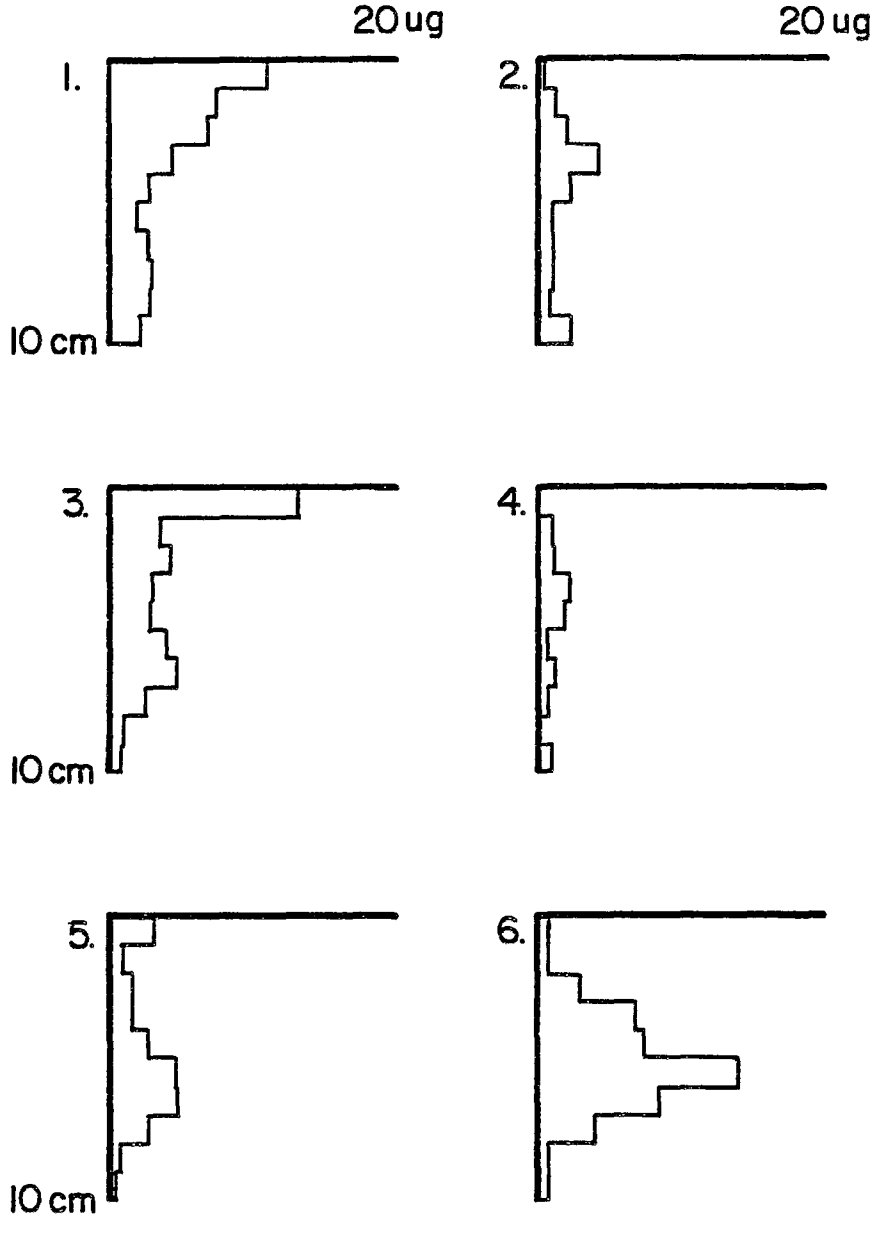


Figure 3.17. -- Concentration of chlorophylls and phaeophytins as a function of depth in sediment.

b. April 1981. Shown are South Slough data for chlorophyll (1.) and phaeophytin (2.), and Isthmus Slough data for chlorophyll (3.), phaeophytin (4.), bacteriochlorophyll (5.), and bacteriophaeophytin (6.).

b.

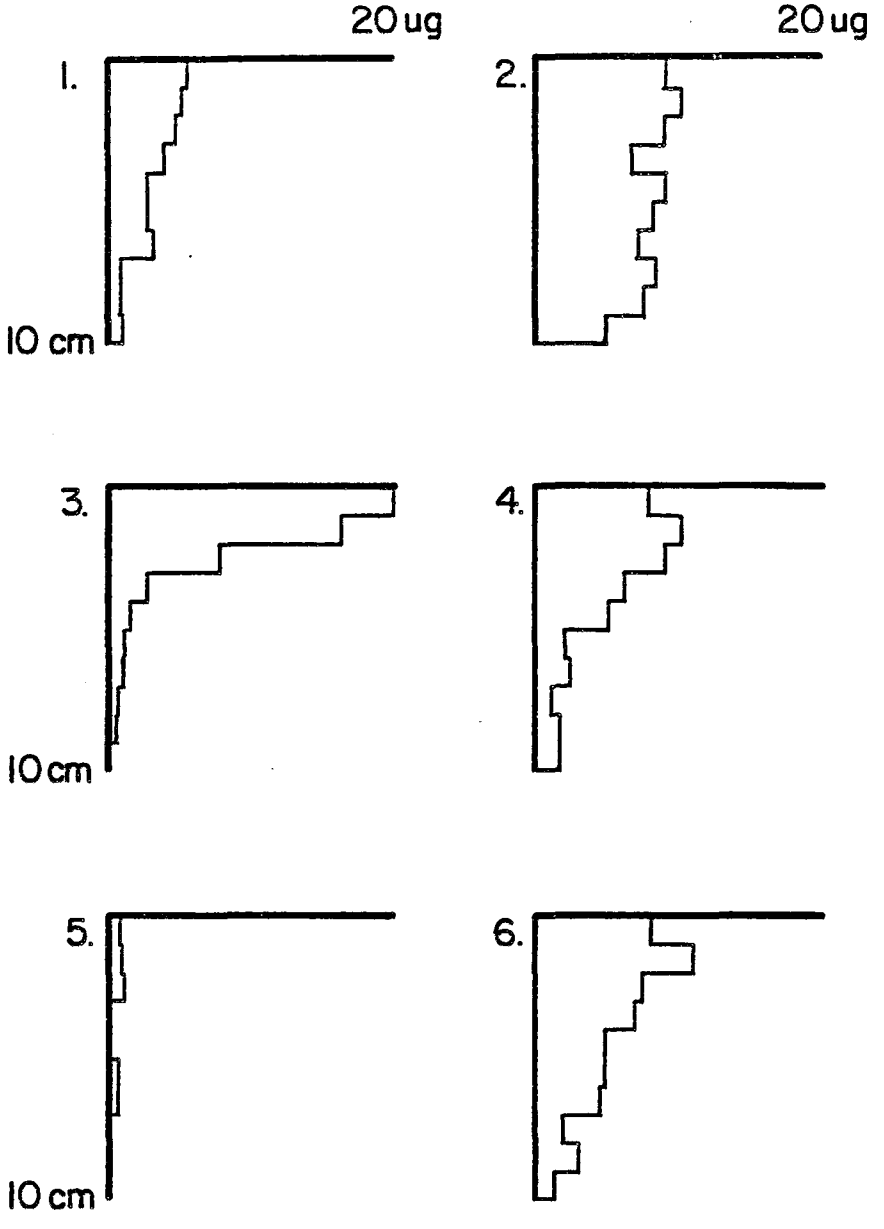


Figure 3.17. -- Concentration of chlorophylls and phaeophytins as a function of depth in sediment.

c. July 1981. Shown are South Slough data for chlorophyll (1.) and phaeophytin (2.), and Isthmus Slough data for chlorophyll (3.), phaeophytin (4.), bacteriochlorophyll (5.), and bacteriophaeophytin (6.).

C.

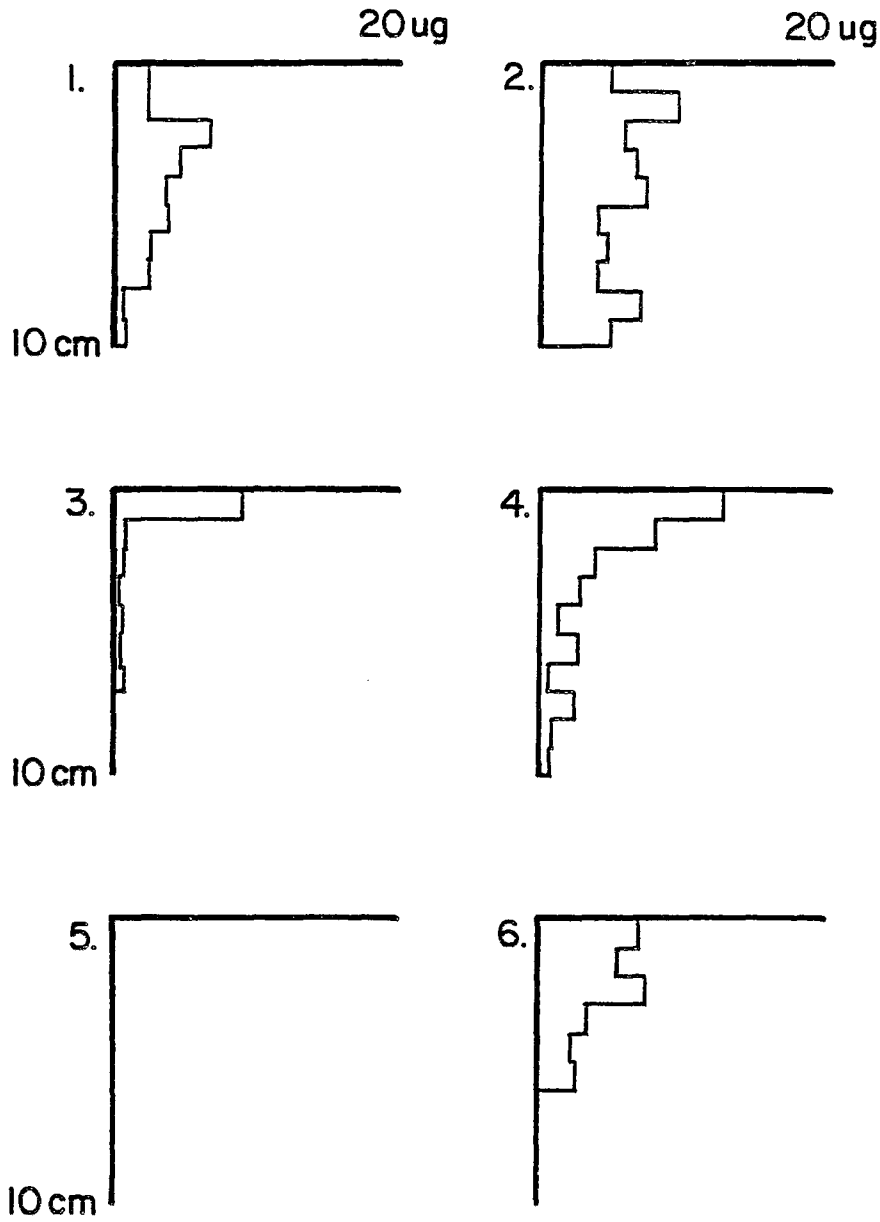
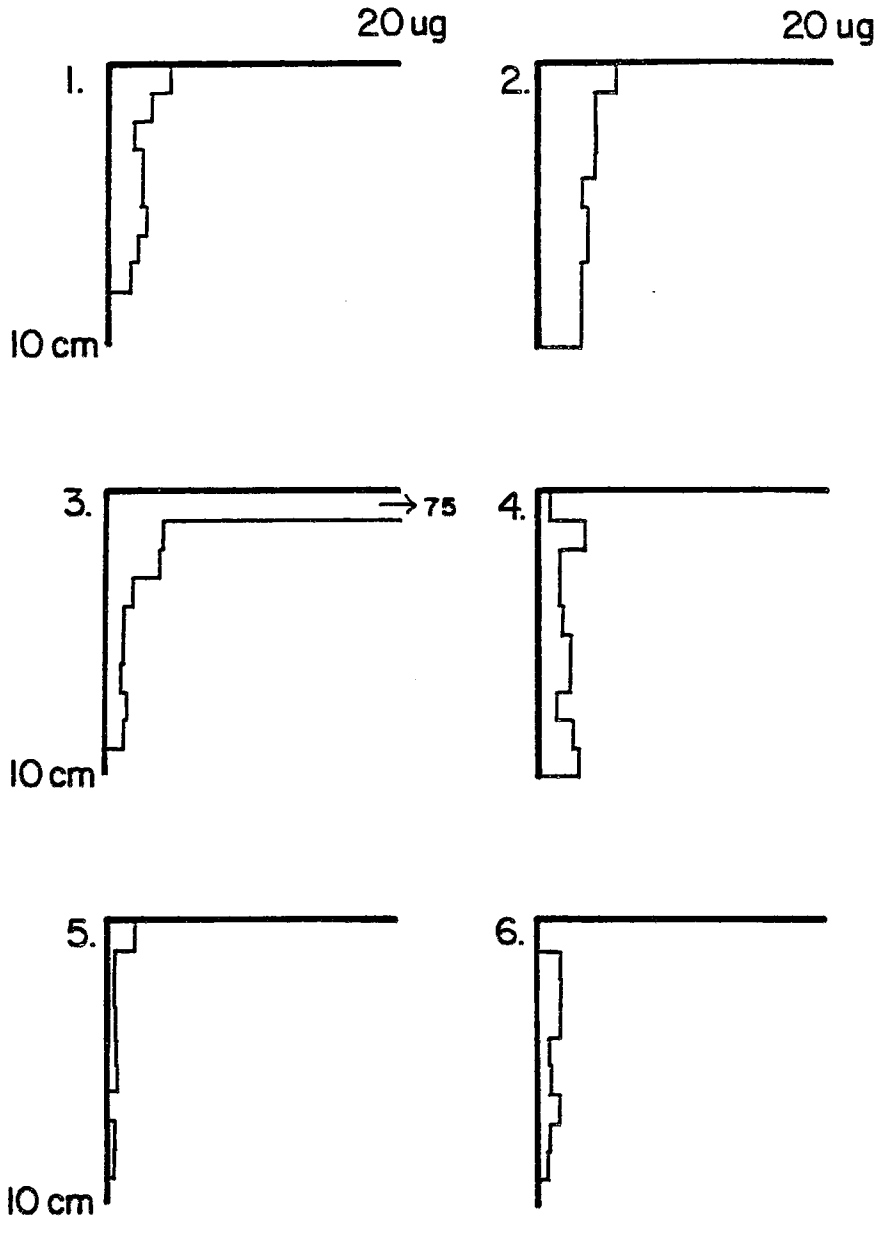


Figure 3.17. -- Concentration of chlorophylls and phaeophytins as a function of depth in sediment.

d. October 1981. Shown are South Slough data for chlorophyll (1.) and phaeophytin (2.), and Isthmus Slough data for chlorophyll (3.), phaeophytin (4.), bacteriochlorophyll (5.), and bacteriophaeophytin (6.).

d.



Discussion

The lack of bacteriochlorophyll at most locations suggests that while exposed mudflats in Coos Bay support an active layer of sulfur reduction, they do not have an active surface layer of sulfur oxidation. The occurrence of an active surface layer of sulfur oxidizers at the unusual location, Isthmus Slough, is due both to the greater input of organic material and to the decreased oxygen penetration into the sediment caused by this fine-grained material. The dike protecting the seaward side of Isthmus Slough's communication with the bay creates a zone of calm water in which organics can settle out, futhermore it allows the annual growth of vascular plants which provides organic matter when plants die in the fall. The vascular plants growing here are typical of Coos Bay salt marshes located higher in relation to the tides, with the exception of Eleocharis which I have only found at Isthmus Slough.

Chlorophyll and phaeophtyin show a gradual decrease in concentration with depth at both locations. This is the distribution one expects from a mixing down from the photosynthetic layer of the surface, followed by gradual decomposition of pigments. During most of the year the major mechanism for mixing is burrowing by invertebrates. Passage of sediment through the gut of these invertebrates would also account for the generation of such large amounts of phaeophytin in the sediment (Currie, 1962).

Bacteriochlorophyll of the purple bacteria may be mixed down by invertebrates, but at least part of the second horizon within Isthmus Slouth sediment is due to growth of cells in situ. Pigments at both horizons are greatest during months when sediment iron has been charged and free sulfide is available, suggesting that growth is in part powered by the oxidation of sulfide by means of molecular oxygen. Oxygen in pore water can be detected within the upper layers of black sediment such as where these cells are growing at Isthmus Slough, even though on a gross scale the eH is negative (Novitsky, Scott, and Kepkay, 1981). It is also possible, however, that these cells are growing anaerobically. Purple bacteria have been shown to grow anaerobically in the dark using compounds common in intertidal mud; this growth being sulfide-dependent in some species (Krasilnikova, Pedan, and Kondrateva, 1976) and not sulfide-dependent in others (Uffen and Wolfe, 1970).

Purple bacteria, like higher plants, make chlorophyll when light intensity increases and stop making chlorophyll when placed in the dark. But purple bacteria can do so only in the absence of oxygen. If oxygen is present they do not make chlorophyll, even in the light. Apparently, the purple bacteria regulate chlorophyll synthesis according to the oxidation/reduction state of some electron carrier associated with photosynthesis (Cohen-Bazire, Sistrom, and Stanier, 1957). When the system is reduced by a supply of light-energized electrons the cell responds by making chlorophyll; when darkness stops the flow of electrons the system drifts towards oxidation and the cell responds by stopping chlorophyll synthesis. But

in the presence of oxygen the system becomes oxidized regardless of light conditions, and during growth in sulfide-rich mud the system becomes reduced regardless of light conditions. Thus, in mud, chlorophyll is produced that will never see the light of day.

CHAPTER FOUR

SULFUR AND CARBON FLUX

South Slough, a typical Coos Bay mudflat, and Isthmus Slough, a location of higher sediment organic content, were studied intensively for one year. Between July 1981 and May 1982, monthly samples were performed for these two locations. Sulfide emission from the sediment to the atmosphere was measured in the field during low tide, sulfide production and carbon dioxide uptake measurements were made in the laboratory using intact sediment cores collected from these two locations. From these measurements of linked sulfur and carbon flow, estimates of energy flow were made.

Methods

Sulfide Production

The rate at which sulfate is respired to sulfide was measured according to the technique of Jorgensen (1977a). Polybutyrate tubing 2.5 cm in diameter was pressed into sediment collected from the sampling locations to a depth of about 4 cm. A rubber stopper was put in the bottom of each tube, and the tubes were placed in a water bath at the temperature of the sediment when it was collected. After a few hours, sulfur-35 as Na_2SO_4 was injected at 2 cm beneath the sediment surface in each of three tubes from each location. After 24

hours, the reaction was stopped by cooling to the temperature of solidified carbon dioxide. The tubes and stoppers were removed from the cores; each core was placed in a separate glass vessel containing 2 molar oxygen-free hydrochloric acid. Nitrogen gas was bubbled into the solution in this vessel and was carried, along with sulfide released from the sediment, through a glass tube into another vessel containing a solution of basic 0.1 molar $CdCl_2$. After one hour of bubbling, a fraction of this solution (containing precipitated sulfide) and a fraction of the acid sulfate solution were removed for determination of radioactivity. Samples were placed in a glass vial containing a toluene-based, water-accepting gelling scintillation cocktail. Activity in each vial was determined using a Beckman Tri-carb Series 3000 Liquid Scintillation Counter. Actual activity was determined by the channels ratio method according to standards prepared in the same type of cocktail.

Sulfide Emission

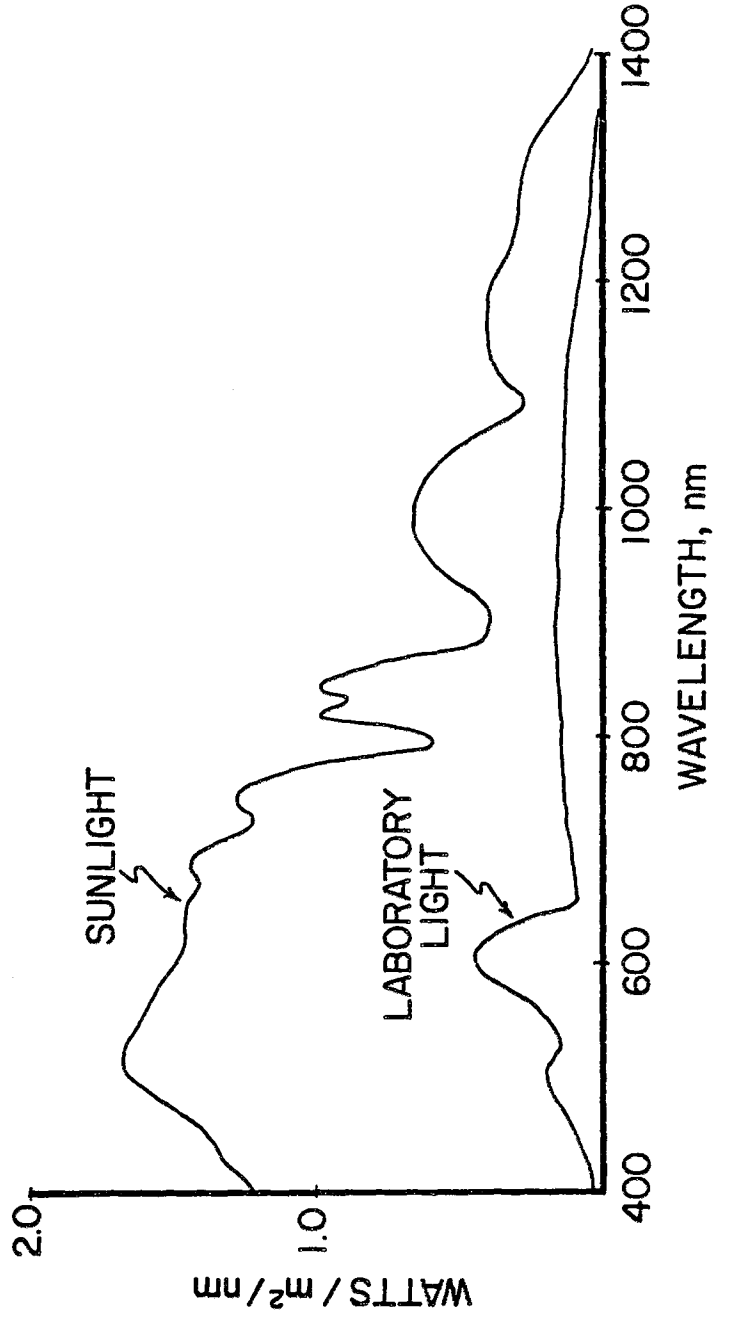
Sulfide loss to the atmosphere was measured by a method similar to that of Hansen (1978). A clear polyacrylate box, 5 cm tall, was pressed halfway into the sediment. Nitrogen from a pressurized tank flowed into an opening on one side of the box. This nitrogen, plus all volatile compounds generated within the box such as sulfide, flowed out an opening on the other side of the box. The gas was bubbled

through a solution of basic 0.1 molar $CdCl_2$. The amount of sulfide precipitated in one hour of gas flow was determined by iodine titration (Golterman, 1971).

Carbon Dioxide Uptake

Uptake of carbon dioxide by organisms at the sediment surface was measured by a technique similar to that of Van Raalte et al. (1974). Shallow cores of sediment were prepared by pressing 2.5 cm diameter polybutyrate tubes into intact sediment, removing the tubes, and then letting all but 1 cm of the core slide out. The sediment was sliced at the base of the tube with a scalpel, then a rubber stopper was put in to hold it. Filtered full-strength seawater with carbon-14 as $NaCO_3$ was poured onto the core (10 ml of water having activity 0.33 μCi/ml) and a layer of petrolatum was poured onto the surface of the water to prevent carbon dioxide in the air and water from mixing. All tubes were placed in a water bath at the temperature of the mudflat when samples were collected in the field. The water bath was situated under a bank of fluorescent and incandescent bulbs giving a light intensity of roughly one-tenth sunlight and a spectral distribution as shown in Figure 4.1. After three hours of incubation, formaldehyde solution was injected beneath the petrolatum layer in order to stop the reaction. Each tube was immediately stripped of its petrolatum layer, and the sediment washed into a glass centrifuge tube. The contents were centrifuged and the supernatant discarded. To each sample was added exactly 10 ml of concentrated nitric acid. Organic

Figure 4.1. -- Light energy supplied by laboratory apparatus compared to that supplied by sunlight, as a function of wavelength. Power units are Watts per square meter illuminated per nm bandwidth; supplied by ten-40 Watt "cool white" fluorescent bulbs and four-100 Watt incandescent bulbs.


SUNLIGHT
LABORATORY LIGHT
2.0
1.0
WATTS / m²/nm
400
600
800
1000
1200
1400
WAVELENGTH, nm

compounds, including carbon-14 labeled ones, were solubilized by the acid. The slow bubbling action produced by the acid stripped the CO_2 that had not been consumed during the experiment, therefore stirring during digestion was not necessary. After 12 hours the contents of each tube was homogenized by stirring, then centrifuged, and the supernatant was sampled. This aliquiot of supernatant was added to a glass vial containing the scintillation cocktail described for Sulfur-35 measurement and the activity measured with a liquid scintillation counter. Corrected activity was determined using the channels ratio method. Carbon fixation was estimated as described by Strickland and Parsons (1972).

For each location, three treatments of three replicates each were performed: incubation with Carbon-14 in the dark, incubation in the light, and incubation in the light with dichlorophenyldimethyl urea (DCMU) present. DCMU stops photosynthesis by algae and vascular plants but allows bacterial photosynthesis to continue. The concentration of DCMU used in this experiment was 10^{-4} molar. This high concentration was used to insure penetration into blades of vascular plants, when present (Forti and Parisi, 1962).

Results

Sulfide Production

Sulfide production at South Slough and Isthmus Slough are shown on Figure 4.2. These data represent the production per square meter of sediment horizon between 1 cm and 2 cm depth, a volume of 10 liters.

Figure 4.2. -- Sulfide production within one-centimeter thick horizon at 2 cm depth.

a. South Slough.

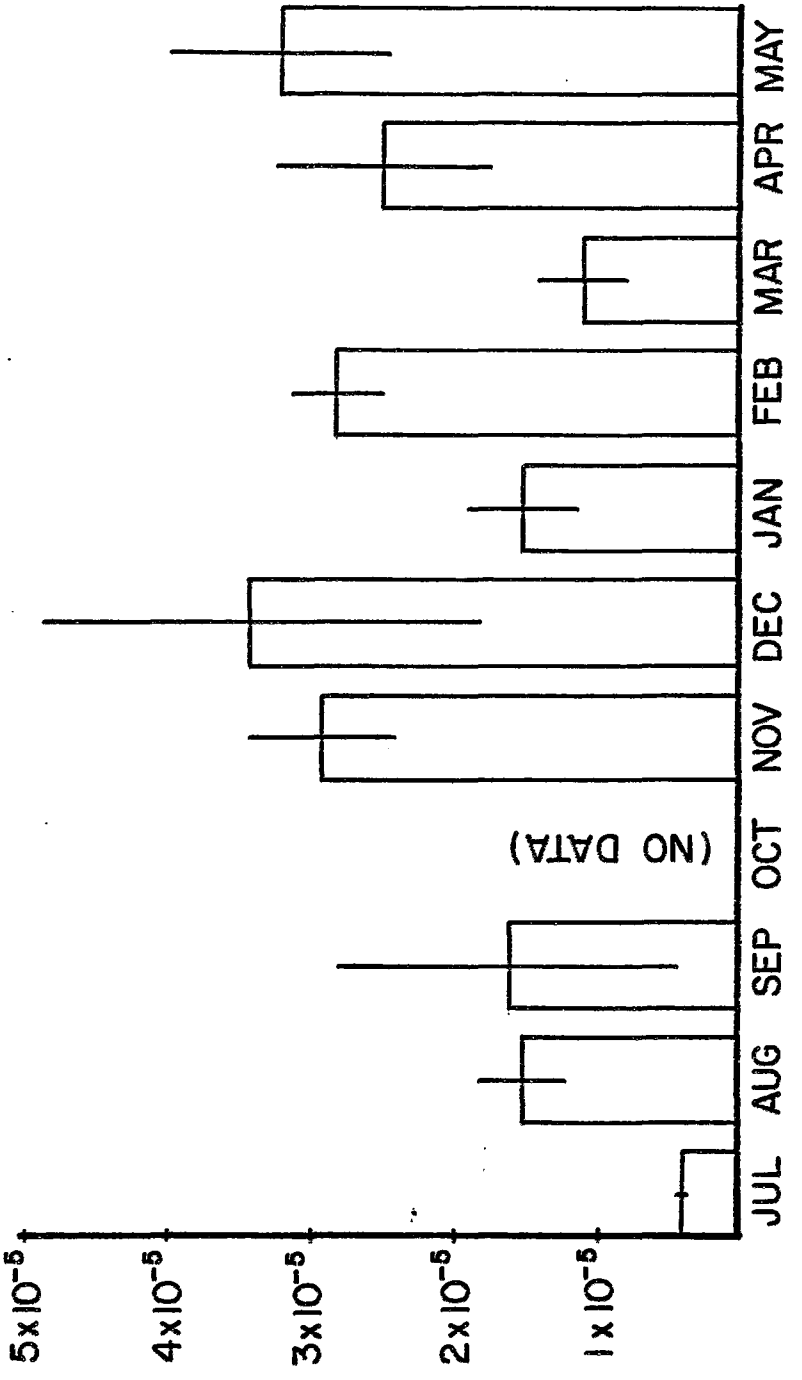
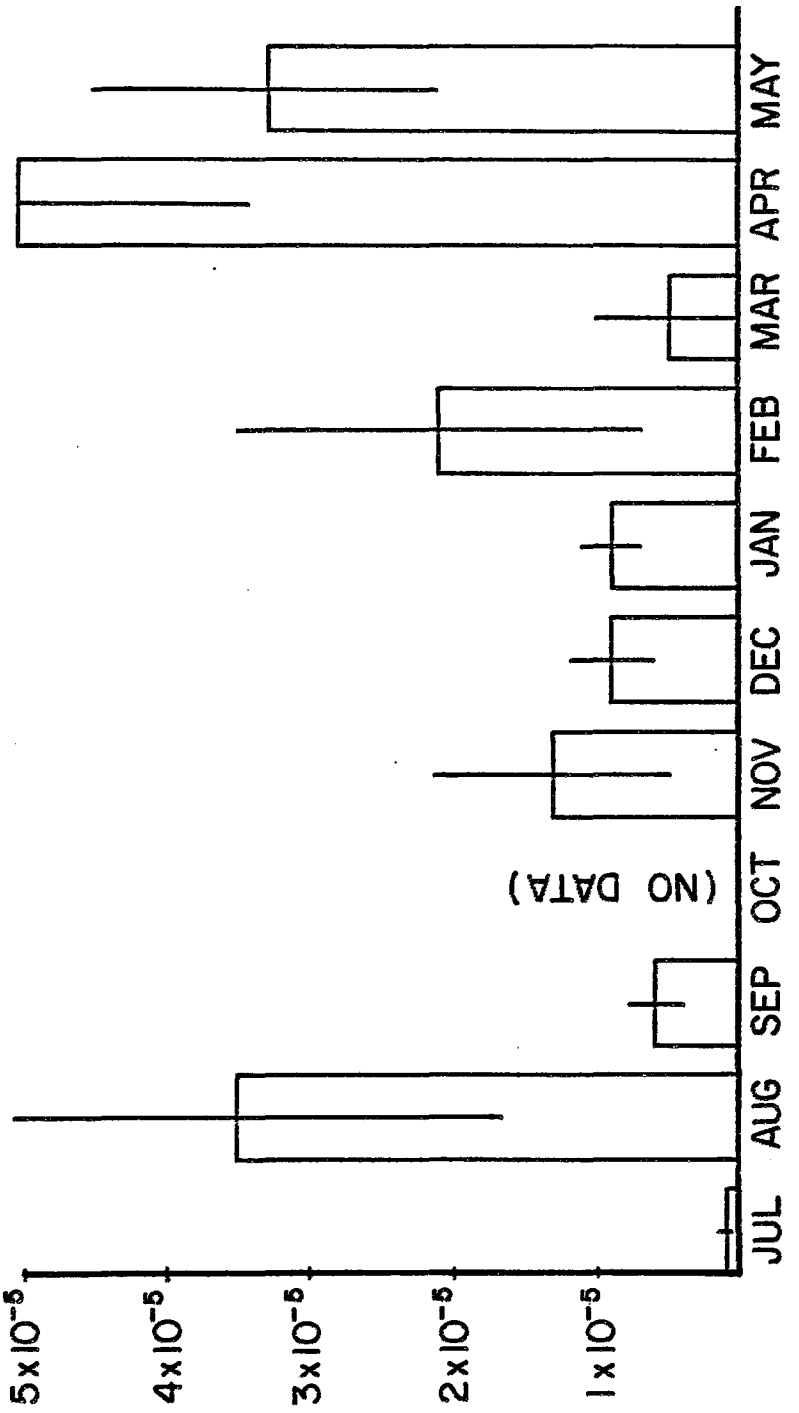

5x10⁻⁵
4x10⁻⁵
3x10⁻⁵
2x10⁻⁵
1x10⁻⁵
JUL
AUG
SEP
OCT
(NO DATA)
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.2. -- Sulfide production within one-centimeter thick horizon at 2 cm depth.

b. Isthmus Slough.


5x10⁻⁵
4x10⁻⁵
3x10⁻⁵
2x10⁻⁵
1x10⁻⁵
JUL
AUG
SEP
OCT
(NO DATA)
NOV
DEC
JAN
FEB
MAR
APR
MAY

During all times of the year at both locations, this horizon was within the brown oxidized layer of sediment situated on the surface. The mean and standard error determined from these replicates are shown for each month.

Sulfide Emission

Sulfide emission to the atmosphere from sediment while exposed at low tide is shown on Figure 4.3. The value of a single determination is shown for each month, precision of the measurements is about 2×10^{-5} moles/m^2/hour.

Carbon Dioxide Uptake

Carbon dioxide uptake at the sediment surface is shown on Figure 4.4. Each months' data are summarized in three bars. Each bar represents the mean and standard error of three replicates. The left bar for each month is uptake in the dark; the middle bar is uptake in the light with DCMU present; the right bar is uptake in the light.

A comparison of carbon dioxide uptake in the laboratory to that in full sunlight is shown on Figure 4.5. Here, cores collected at Isthmus Slough in February were exposed to the standard laboratory lighting and to noon-time sunlight on a cloudless day. The values for each treatment under the two conditions are shown in the same form as the previous two Figures.

Figure 4.3. -- Sulfide emission to the atmosphere.

a. South Slough.

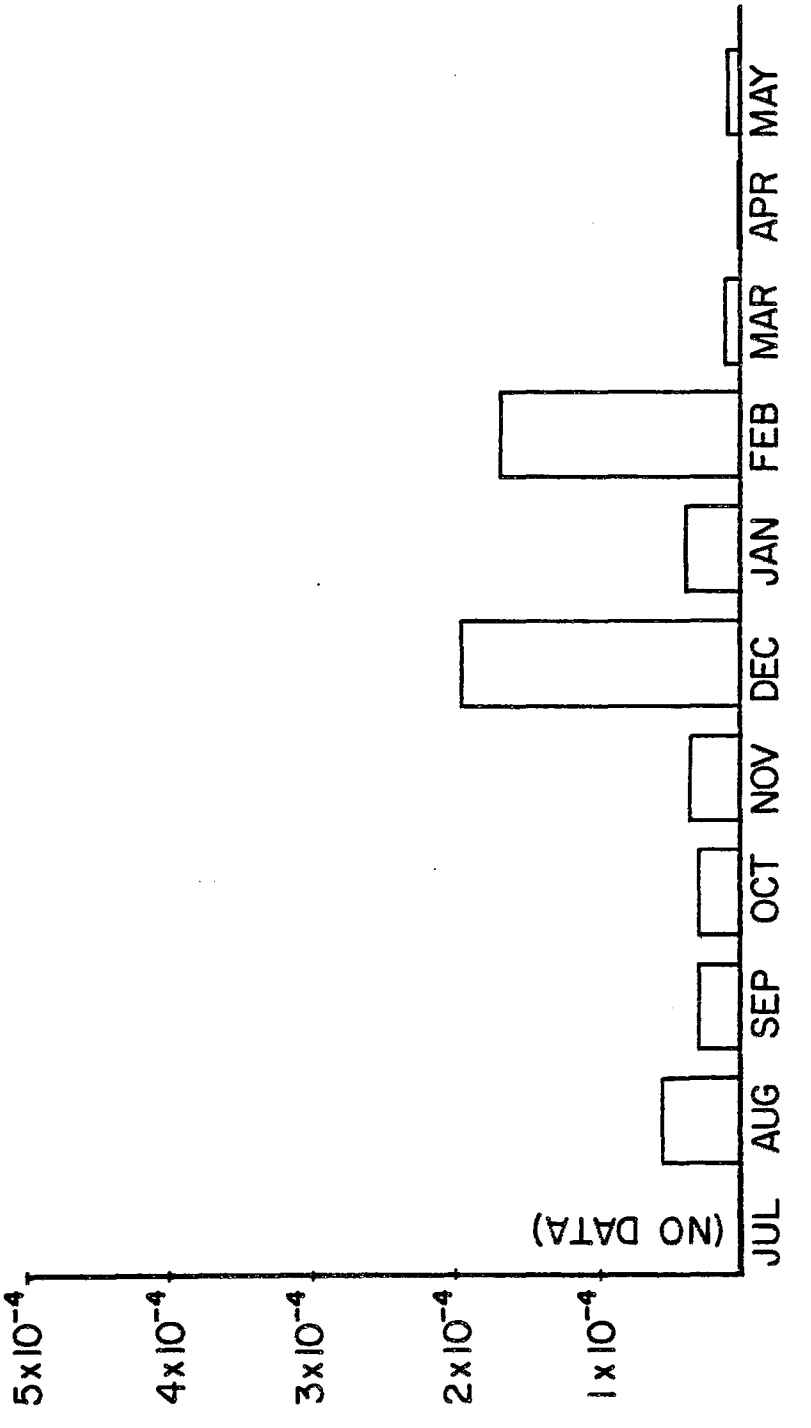

5x10⁻⁴
4x10⁻⁴
3x10⁻⁴
2x10⁻⁴
1x10⁻⁴
(NO DATA)
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.3. -- Sulfide emission to the atmosphere.

b. Isthmus Slough.

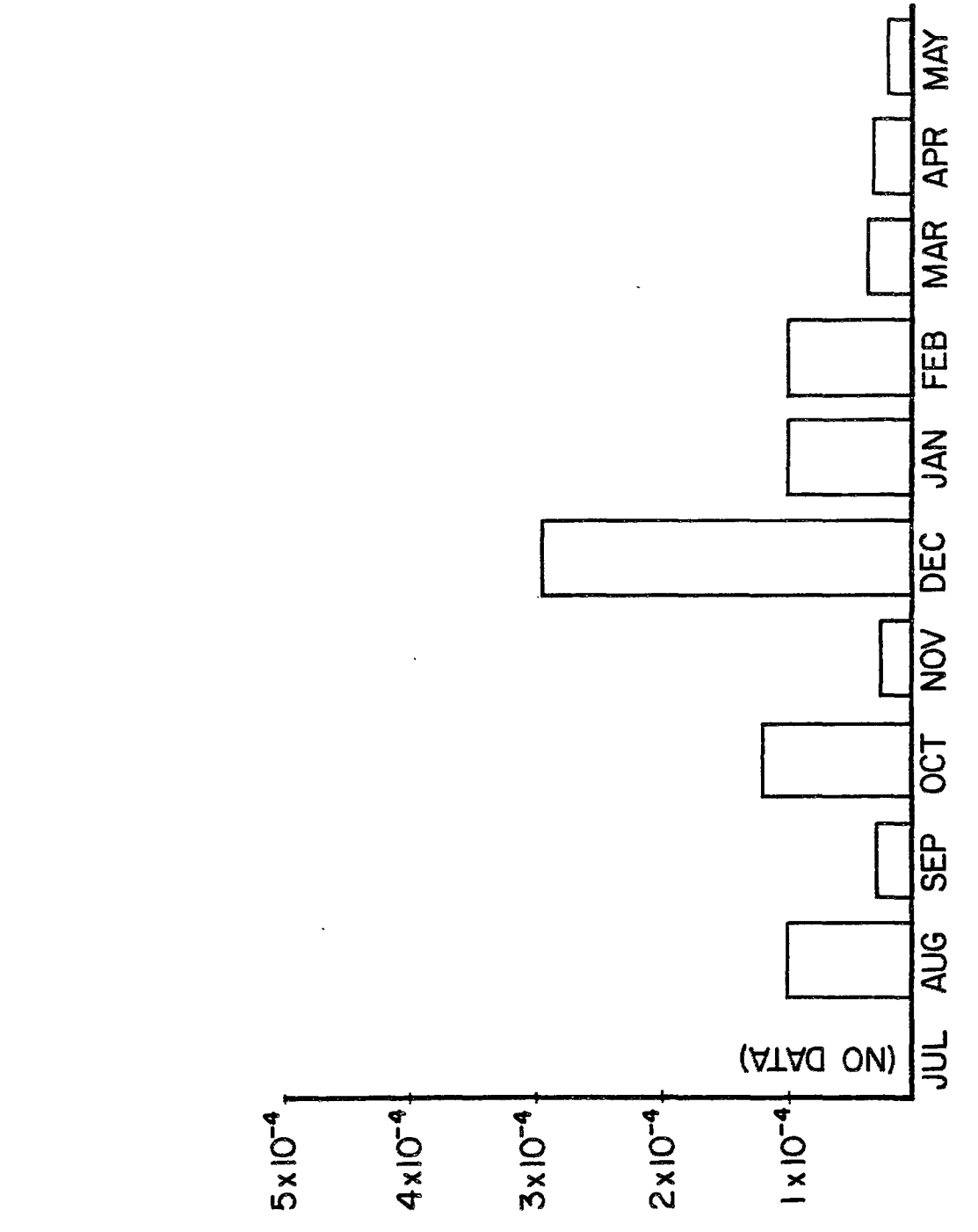

5x10⁻⁴
4x10⁻⁴
3x10⁻⁴
2x10⁻⁴
1x10⁻⁴
(NO DATA)
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.4. -- Carbon dioxide uptake by surface sediment.

a. South Slough.

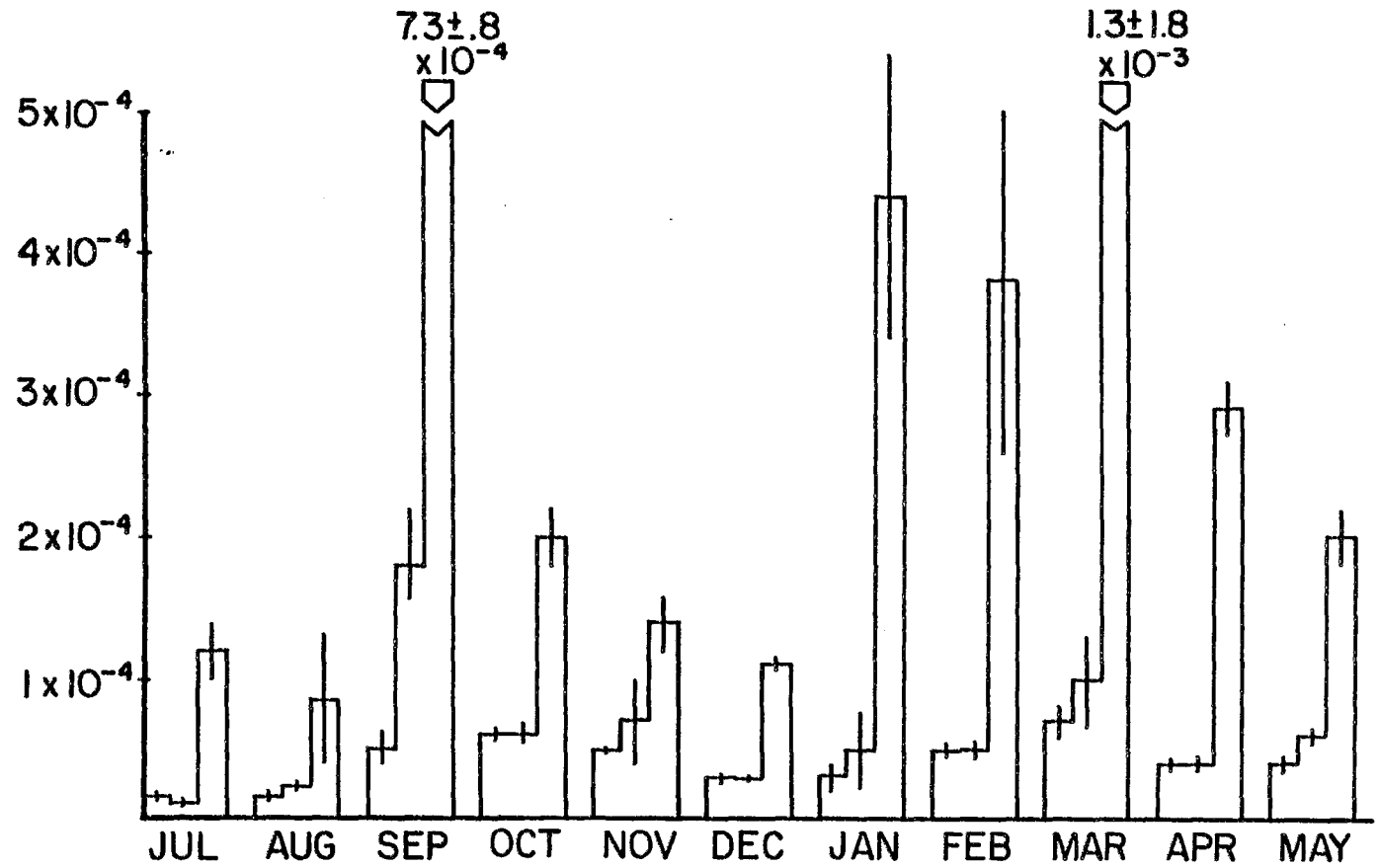

7.3±.8
x10⁻⁴
1.3±1.8
x10⁻³
5x10⁻⁴
4x10⁻⁴
3x10⁻⁴
2x10⁻⁴
1x10⁻⁴
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.4. -- Carbon dioxide uptake by surface sediment.

b. Isthmus Slough.

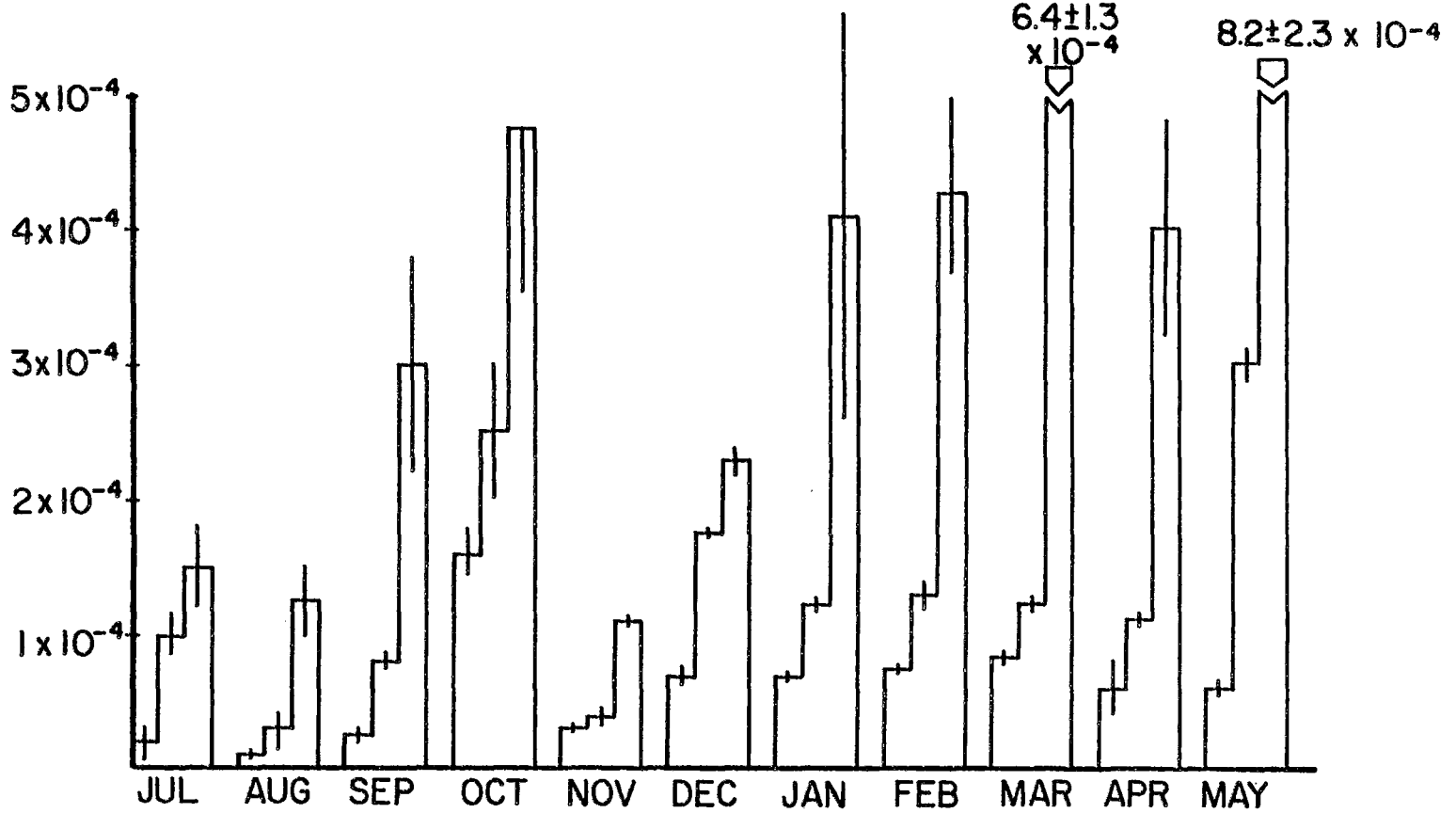
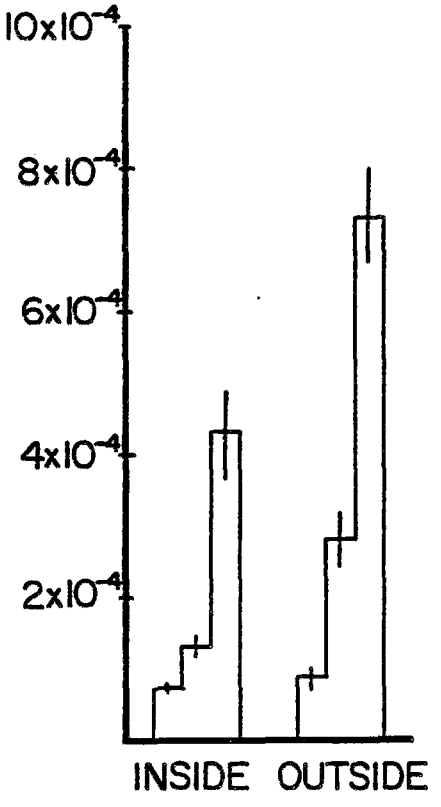

6.4±1.3
x 10-4
8.2±2.3 x 10-4
5x10-4
4x10-4
3x10-4
2x10-4
1x10-4
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.5. -- Carbon dioxide uptake by surface sediment in laboratory light compared to uptake in sunlight. Sediment collected at Isthmus Slough in February 1982.


10x10⁻⁴
8x10⁻⁴
6x10⁻⁴
4x10⁻⁴
2x10⁻⁴
INSIDE
OUTSIDE

Hourly carbon dioxide uptake, corrected by these factors is shown on Figure 4.6. Plotted are nonphotosynthetic uptake (dark treatment), photosynthetic bacterial uptake (difference between dark treatment and DCMU/light treatment), and photosynthetic plant uptake (difference between DCMU/light treatment and light treatment). No statistically significant amount of photosynthesis by bacteria occurred at South Slough.

Values of carbon dioxide uptake per year referred to in Chapter Five are hourly values corrected by these factors and then multiplied by the number of daylight hours sediment at this tidal height is exposed during each day. The average number of hours of daylight exposure in each of the months was computed by Marshall Pregnall (personal communication) from published tide data for the Oregon coast.

Discussion

Sulfide Production

Studies by other workers show that sulfide production is strongly temperature dependent. Production increases linearly as temperature increases over the temperature range encountered in nature (Abdollahi and Nedwell, 1979). In general, incubation temperatures used in this study were higher from July to December than from January to June, since the temperature measured during collection of samples at low tide was higher in the last half of the year when low tide occurs

Figure 4.6. -- Estimated carbon dioxide uptake by surface sediment.

a. South Slough sampling location.

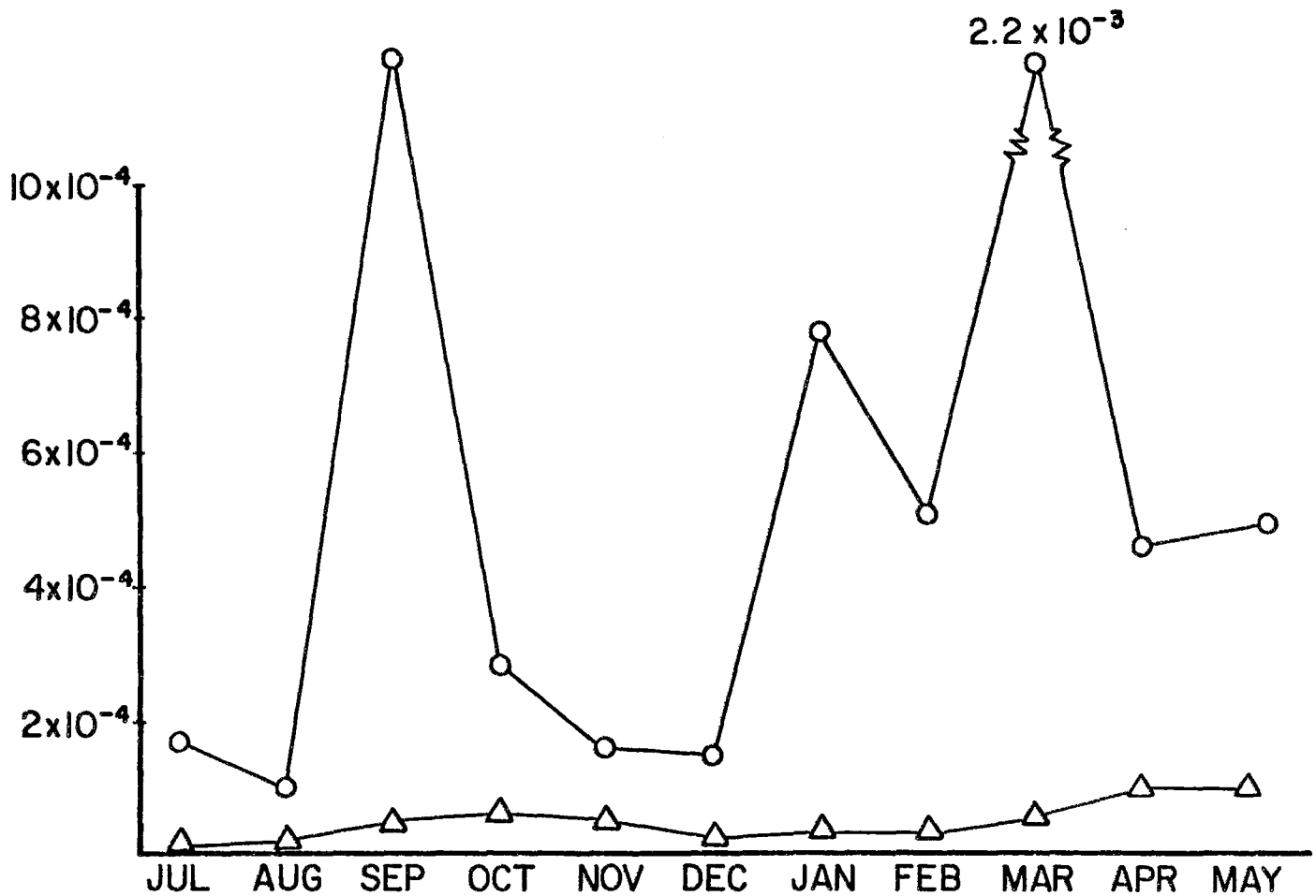
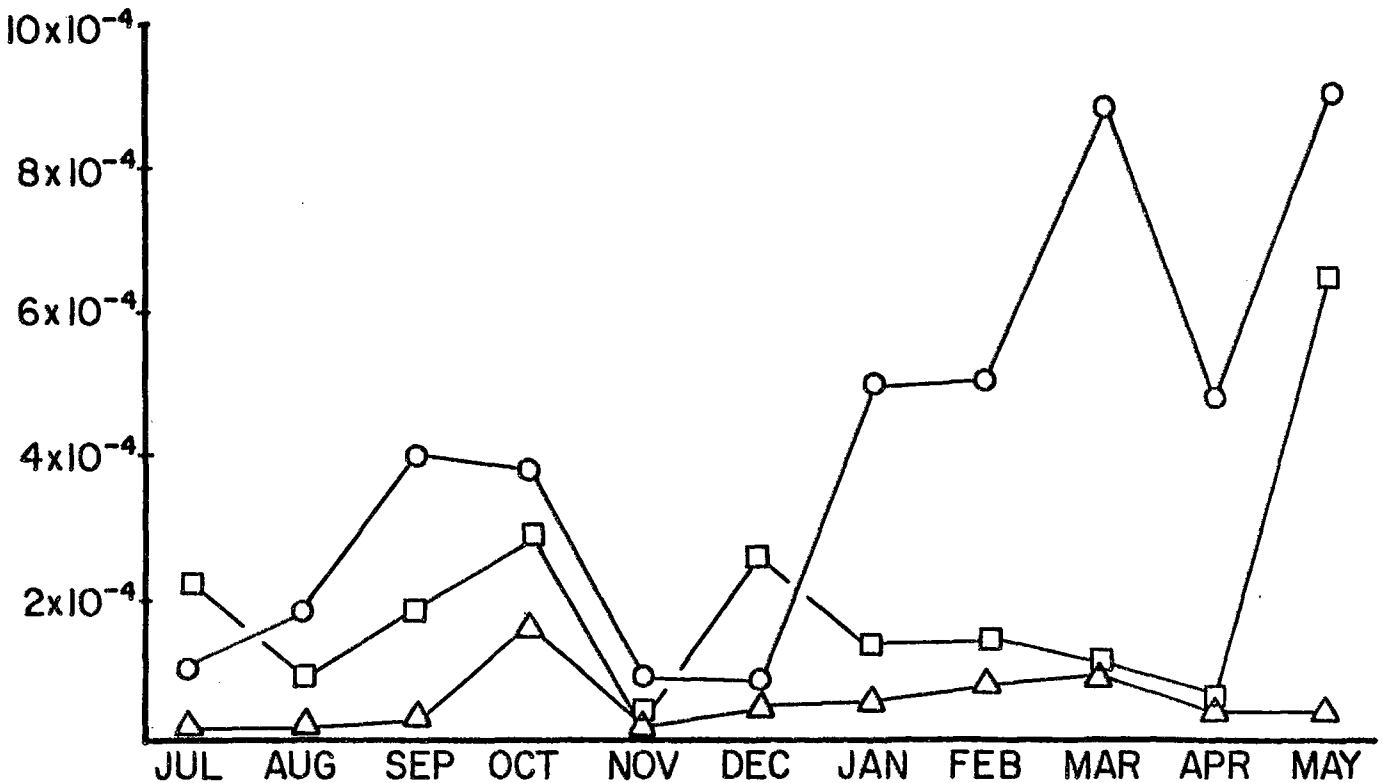

2.2 x 10⁻³
10x10⁻⁴
8x10⁻⁴
6x10⁻⁴
4x10⁻⁴
2x10⁻⁴
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

Figure 4.6. -- Estimated carbon dioxide uptake by surface sediment.

b. Isthmus Slough sampling location.


10x10⁻⁴
8x10⁻⁴
6x10⁻⁴
4x10⁻⁴
2x10⁻⁴
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY

usually during daylight hours than during the first half of the year when low tides usually come at night.

In the field, annual variation in rate of organic matter input is superimposed on variation in temperature. Thus sulfide production is higher in autumn and lower in spring than would be predicted from temperature alone (Howarth and Teal, 1979).

A graph of sulfide production at any time of the year as a function of depth within intertidal sediment is a curve with maximum rate at about a decimeter of depth. It approaches zero towards the surface and has a more gradual drop-off downwards. A study by Jorgensen (1977a) shows that the magnitude of the peak and gradients in each direction are largest in late autumn and early winter. Determination of an accurate profile requires a tremendous number of samples; such a profile was not done for this study. A rough lower estimate of sulfide production in the upper 20 cm of sediment, which accounts for nearly all production, is made by raising the value at 2 cm by one order of magnitude.

At the 2 cm horizon, production of sulfide for the year at Isthmus Slough is lower than that at South Slough. This is because of the depletion of sulfate during the winter when water overlying the upstream Isthmus Slough location is made brackish by increased rainfall.

Sulfide Emission

Unexpectedly large amounts of sulfide were emitted by sediment. The rates at Isthmus Slough were within published ranges for estuarine mud of such high organic content (Adams et al., 1981; Hansen, 1978). Rates at South Slough were lower than Isthmus Slough but higher than those of similar exposed mud in Atlantic estuaries. Yet, seldom can one smell sulfide at South Slough and purple bacteria indicative of sulfide are never found in large numbers. It is possible that forms of reduced sulfur other than sulfide are being measured by this technique. These would include compounds known to be emitted from mudflats: carbonyl sulfide and methyl mercaptan (Adams et al., 1981), dimethyl sulfoxide (Andreae, 1980), carbon disulfide (Lovelock, 1974), and dimethyl sulfide (Lovelock, Maggs, and Rasmussen, 1972). Such compounds may be retained by the cadmium in the measurement apparatus but not by iron in the sediment, thus explaining the absence of the characteristic black color of reduced sediment. It is also possible that sulfide reaching the atmosphere is not passing through the oxidized layer but is produced within a microlayer at the sediment surface.

Carbon Fixation

These data represent potential photosynthesis under special laboratory conditions and thus data from different months are directly comparable. The decrease in specific activity of C-14 in water

immediately over the sediment, which is due to uptake and to addition of nonradioactive carbon by respiration, is not taken into account. Thus, values are lower estimates (Revsbech, Jorgensen, and Brix, 1981).

Approximate conversion to daylight values can be made using the data on Figure 4.5. In these experiments, photosynthesizers seem to approach saturation at far less than daylight intensities. Both bacterial and algal photosynthesis increase only by about a factor of two. Although one might expect higher intensities outdoors to inhibit bacterial photosynthesis via increased oxygen production by algae, the factor of increase is higher for bacteria than for algae plus vascular plants. A reasonable interpretation is that bacteria, which because of their use of sulfide are found underneath the diatoms (Parkin and Brock, 1980) benefit from increased light penetration.

CHAPTER FIVE

SUMMARY

The seasonality in physical sediment characteristics, such as oxygen uptake, sulfate and sulfide concentrations in pore water, and the fraction of iron which is bound to sulfur, described for northeast Pacific estuaries by other workers (Bella, 1975; Crook, 1970; Pamatmat, 1968) is reflected here in direct measurements of sulfur cycle bacteria and their activity in the sediment. Sulfide from anaerobic respiration is produced in greatest quantities during the fall and winter; sulfide emission to the atmosphere and sulfide uptake by photosynthetic bacteria peak in the late fall. Spring and summer are times of diminished sulfide production and consumption.

The annual cycle in sedimentary sulfide content is buffered by iron, which can reversibly bind sulfide. Iron dissolved in river water precipitates in the salt water of the estuary and is buried, hence, iron is abundant in the sediment. During winter months iron discharges its sulfide as that in pore water is oxidized; during summer months it is recharged and by late fall sulfide production in the sediment is nearly all available for use by bacteria. This annual charging and discharging is visible to the casual observer as a yearly rise and fall in the depth within the sediment of the black reduced iron layer which is characteristic of sulfide-producing mud.

In early spring the brown layer, which contains oxidized iron, overlying the black layer is a decimeter thick. By fall it shrinks to little more than a centimeter.

An Energy Budget of Intertidal Sediment

From measured rates of sulfur and carbon flux, estimates of energy flow can be made. The appropriate unit for energy flow is the Watt (W), which is equal to one joule per second. Data from this study, in units of moles/m^2/h, were transformed using the actual free energy change values calculated by Howarth and Teal (1980) to give Watts per square meter.

South Slough

Taking into account the change in daylight hours and time of low tides during the year, one square meter of Coos Bay mud at +1 meter elevation is receiving sunlight at an annual average rate of about 100 W/m^2. This value assumes clouds to be absent and thus it is an underestimate.

Only a fraction of this energy is used in photosynthetic carbon fixation. The 0.94 moles/m^2/year carbon fixed at South Slough represents an annual average energy flow of 0.015 W/m^2.

If all of this carbon created *in situ* were respired by sulfide-producing bacteria, it would account for 0.47 moles/m^2/year. The actual rate is 1.67 moles/m^2/year, which is four times greater. This

sulfide, if moved to oxygenated layers and oxidized completely using molecular oxygen, would correspond to a free energy flow of 0.039 W/m^2.

The loss of sulfide to the atmosphere at low tide, 0.19 moles/m^2/year, represents an average energy flow of 0.004 W/m^2. If diffusion of sulfide is the rate limiting step, then an equal amount is lost to the water at high tide. This amounts to one-fifth of that estimated to be produced deep within the sediment.

Reduced sulfur compounds may be incorporated permanently into sediment minerals, or they may leach from the sediment at low tide. Neither of these rates was measured in this study.

Isthmus Slough

At Isthmus Slough, carbon dioxide uptake was measured to be 1.34 moles/m^2/year. Averaged over the year, this represents 0.021 W/m^2.

If all of this carbon were consumed by sulfide-producing bacteria, 0.74 moles/m^2/year would be created. The actual sulfide production is 1.57 moles/m^2/year, which corresponds to 0.037 W/m^2.

Emitted to the atmosphere was 0.34 moles/m^2/year of sulfide. This, plus an equal loss to water at high tide, corresponds to 0.016 W/m^2. It is half the estimated production within the sediment.

Some sulfide was also consumed by photosynthetic bacteria. If all electrons for photosynthesis in these bacteria were supplied by sulfide and none were supplied by organic compounds, the sulfide

consumed would represent 0.006 W/m^2. This is one-third of that leaving the sediment.

Conclusions

Sulfide Production

The amount of sulfide produced at South Slough and Isthmus Slough is similar to that reported in other studies of intertidal mud (Abdollahi and Nedwell, 1979) but lower than that of salt marshes (Howarth and Teal, 1979) and Scandinavian fjords (Jorgensen, 1977a).

Assuming that the photosynthetic productivity of water overlying intertidal sediment is as great as that of the sediment (Joint, 1978), then nearly enough reduced carbon would be produced at both Isthmus Slough and South Slough to support this sulfide production. The studies by Howarth and Teal (1980) and Jorgensen (1977a) indicate, however, that half the sedimentary breakdown of organic matter is by oxygen respiration. This suggests an input of organic matter to the sediment as great as the amount produced in situ. This additional organic input could be from the settling of detritus out of the water column, or from the accumulation by invertebrates actively filtering the water column.

For each square meter of sediment surface at South Slough and Isthmus Slough, the flow of energy into reduced sulfur is twice that flowing into reduced carbon. However, sulfide is unlike organic matter

in the sense that it is quite unstable outside of the anoxic mud and will spontaneously oxidize if it is not first used biologically. In this study the rate of sulfide oxidation deep within the sediment is not measured; hence the amounts of energy stored in sulfur and carbon compounds cannot be compared.

Sulfide Leaving the Sediment Surface

The amount of sulfide reaching the atmosphere from South Slough, a location typical of Coos Bay, is similar to that measured for high-organic content Atlantic intertidal sediment (Adams et al., 1981) and fjord sediment covered by a few centimeters of water (Hansen, 1978). Yet this amount, plus an estimated equal amount lost to the water during high tide, is more than one-fifth the total estimated production within the sediment. At Isthmus Slough, the comparable fraction is one-half. It is unlikely that such a large fraction would escape incorporation into iron minerals and loss to chemical and biological oxidation. Furthermore, the measured sulfide concentration in the brown surface layer of mud is zero. A reasonable explanation for this high emission is that in addition to the production of sulfide in the anaerobic layer of sediment, there is significant production in a thin surface layer. The surface is rich in plants and animals which excrete large amounts of reduced carbon which could power sulfate reduction.

Sulfate respiration in the well-oxygenated upper few centimeters has been demonstrated (Goldhaber et al., 1977; Jorgensen,

1977b), but a thin layer with very high production has not been described in the literature. This layer might consist of scattered fecal pellets and other particles rich in organic matter. Jorgensen demonstrated that sulfide could be generated in particles less than 1 mm in diameter and that the time required for a sulfide molecule to reach the outside of the particle would be less than one second. For this present study, several measurements were made of the transfer of sulfur-35 as sulfate into sulfide in water overlying sediment cores, but only trace amounts were found. Assuming that the technique used (Fenchel and Jorgensen, 1977) is accurate under these conditions, then in submerged sediment there must be a very high turnover of sulfate to sulfide, and then finally, to some sulfur compound not measured using this experimental technique. The instantaneous concentration of sulfide would be very low. Considering that oxidation of sulfide in oxygenated seawater takes minutes or hours (Chen and Morris, 1972; Cline and Richards, 1969), some special biological or chemical property of the sediment/water interface would have to be invoked. Such sulfide production would be measurable only when stripped and trapped continuously as it was emitted from sediment not covered by water. The existence of some kind of surface layer is supported by the observation that at Isthmus Slough bacterial photosynthesis varies roughly with measured sulfide emission to the atmosphere, but not with measured sulfide production.

Whatever the source, large amounts of sulfide enter the atmosphere from Coos Bay intertidal sediment. Using South Slough data as

average values, an estimate of Coos Bay sulfide emission would vary over the year between one-fourth metric ton per day and two metric tons per day.

Bacterial Sulfide Consumption

Carbon dioxide uptake by unilluminated surface sediment, of which some unknown fraction is due to nonphotosynthetic sulfide-consuming bacteria, is much less than uptake by illuminated sediment. The highest value is in October, and may correspond to a peak in nonphotosynthetic sulfide consumption. But in general, the magnitude of uptake in the dark roughly varies with that by algae in the light. This suggests that most measured uptake in the dark is residual carbon dioxide uptake by previously illuminated photosynthesizers.

Total nonphotosynthetic carbon dioxide fixation due to sulfide consumption in the sediment column cannot be estimated since the depth of penetration of the carbon-14 label into the sediment is unknown. The maximum uptake is known to occur at the boundary between the upper oxygen-rich layer and the lower sulfide-rich layer (Kepkay, Cooke, and Novitsky, 1979; Kepkay and Novitsky, 1980) which occurs at several centimeters depth in Coos Bay sediment.

At South Slough, there is no statistically significant difference between carbon dioxide uptake in the dark and that in the light in the presence of DCMU except during the months of September and May. Since no bacteriochlorophyll was detected in South Slough sediment

collected in these months, it is likely that this difference reflects experimental variation rather than bacterial photosynthesis.

Photosynthetic carbon dioxide fixation by bacteria at Isthmus Slough is significant. One-third of the annual fixation is due to bacteria. From month to month this fixation varied roughly with measured sulfide emission rates, suggested that this metabolism is largely sulfide-dependent. However, uptake powered by heterotrophic carbon metabolism in these bacteria is not stopped by the DCMU added in the one treatment. If all measured bacterial photosynthesis is assumed to be sulfide-dependent, the amount of sulfide consumed during the year is only one-third of that leaving the sediment.

Unmeasured Rates

Three mechanisms of sulfide loss have not been accounted for in this study. In increasing probable magnitude, they are: 1) loss by leaching at low tide, 2) incorporation into minerals, and 3) chemical and biological oxidation deep within the sediment.

Much of the sulfide may be partially oxidized, for example to thiosulfate (Howarth and Teal, 1980), and leach into water channels at low tide. At least some estuarine locations have measurable amounts of reduced sulfur in the water and contain bacteria capable of using this reduced sulfur (Howarth and Teal, 1980; Tuttle and Jannasch, 1973).

A very large fraction of sulfide produced within intertidal sediment is, for a time, recoverable as pyrite (FeS_2). Most of this

pyrite is re-oxidized, but the amount remaining may represent 10% of the sulfide produced (Jorgensen, 1977a; Howarth and Teal, 1979).

Sulfide oxidized deep within the sediment is undoubtedly the largest unmeasured rate in this study. Chemical oxidation, and most biological oxidation, occurs by reaction of reduced sulfur compounds with molecular oxygen. Oxygen is known to be present in pore water deep within the sediment. Direct measurement in intact sediment of sulfide oxidation and the carbon fixation which may accompany it is difficult, but it is the one aspect of intertidal sulfur cycling that is currently estimated only by deduction.

BIBLIOGRAPHY

Abdollahi, H., and D. B. Nedwell. 1979. Seasonal temperature as a factor influencing bacterial sulfate reduction in a salt marsh sediment. Microb. Ecol. 5: 73-79.

Adair, F. W., and K. Gundersen. 1969. Chemoautotrophic sulfur bacteria in the marine environment. I. Isolation, cultivation, and distribution. Can. J. Microbiol. 15: 345-353.

Adams, D., et al. 1981. Biogenic sulfur strengths. Environ. Sci. Tech. 15: 1493-1496.

Andreae, M. O. 1980. Dimethylsulfoxide in marine and freshwaters. Limnol. Oceanogr. 25: 1054-1063.

Ansbaek, J., and T. H. Blackburn. 1980. A method for the analysis of acetate turnover in a coastal marine sediment. Microb. Ecol. 5: 253-264.

Bella, D. 1975. Tidal flats in estuarine water quality analysis. E. P. A. Ecological Research Series, Number EPA-660/3-75-025.

Berner, R. A. 1970. Sedimentary pyrite formation. Am. J. Sci. 268: 1-23.

Blackburn, T. H., P. Kleiber, and T. Fenchel. 1975. Photosynthetic sulfide oxidation in marine sediments. Oikos 26: 103-108.

Boaden, P. J. S. 1975. Anaerobiosis, meiofauna and early metazoan evolution. Zool. Scripta 4: 21-24.

Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill. 1977. Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. 33: 1162-1169.

Cammen, L. M. 1980. The significance of microbial carbon in the nutrition of the deposit feeding polychaete Nereis succinea. Mar. Biol. 61: 9-20.

Castenholz, R. 1977. The effect of sulfide on the blue-green algae of hot springs. II. Yellowstone National Park. Microb. Ecol. 3: 79-105.

Cavanaugh, C. M. et al. 1981. Prokaryotic cells in the hydrothermal vent tube worm Riftia pachyptila Jones: possible chemoautotrophic symbionts. Science 213: 340-341.

Chen, K. Y., and J. C. Morris. 1972. Kinetics of oxidation of aqueous sulfide by O2. Environ. Sci. Tech. 6: 529-537.

Cline, J. D., and F. A. Richards. 1969. Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature, and pH. Environ. Sci. Tech. 3: 838-843.

Cohen, Y., E. Padan, and M. Shilo. 1975. Facultative anoxygenic photosynthetis in the cyanobacterium Oscillatoria limnetica. J. Bact. 123: 855-861.

Cohen-Bazire, W. R. Sistrom, and R. Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Phys. 49: 25-51, 56-68.

Crook, G. R. 1970. In situ measurement of the benthal oxygen requirements of tidal flat deposits. Thesis, Oregon State University.

Currie, R. I. 1962. Pigments in zooplankton faeces. Nature 193: 956-957.

Dickerson, R. 1980. Cytochrome c and the evolution of energy metabolism. Sci. Amer. 242(2): 136-153.

Felbeck, H., J. J. Childress, and G. N. Somero. 1981. Calvin-Benson cycle and sulfide oxidation enzymes in animals from sulfide-rich habitats. Nature 293: 291-293.

Fenchel, T. 1969. The ecology of marine microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia 6: 1-182.

Fenchel, T., and R. J. Riedl. 1970. The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. Mar. Biol. 7: 255-268.

Fenchel, T., and B. J. Straarup. 1971. Vertical distribution of photosynthetic pigments and penetration of light in marine sediments. Oikos 22: 172-182.

Forti, G., and B. Parisi. 1962. Evidence for the occurrance of cyclic phosphorylation in vivo. Biochem. Biophys. Acta 71: 1-6.

Friend, J. P. 1973. The global sulfur cycle, pp. 177-201 in S. I. Rasool (ed.) Chemistry of the lower atmosphere. Plenum Press.

Fuller, R. C. 1978. Photosynthetic carbon metabolism in the green and purple bacteria, pp. 691-705 in R. C. Clayton and W. R. Sistrom (eds.) The Photosynthetic Bacteria. Plenum Press.

Gallardo, V. 1977. Large benthic microbial communities in sulfide biota under Peru-Chile subsurface countercurrent. Nature 268: 331-332.

Gargas, E. 1970. Measurements of primary production, dark fixation and vertical distribution of the microbenthic algae in the Oresund. Ophelia 8: 231-233.

Goldhaber, M. B., et al. 1977. Sulfate reduction, diffusion, and bioturbation in Long Island Sound sediments: report of the FOAM group. Am. J. Sci. 277: 193-237.

Golterman, H. (ed.) 1971. Methods for Chemical Analysis of Freshwaters. I. B. P. Handbook Number 8. Blackwell.

Hansen, M. H., K. Ingvorsen, and B. B. Jorgensen. 1978. Mechanisms of hydrogen sulfide release from coastal marine sediments to the atmosphere. Limnol. Oceanogr. 23: 68-76.

Hansen, T. H., and H. van Gemerdan. 1972. Sulfide utilization by purple nonsulfur bacteria. Archiv. Mikrobiol. 86: 49-56.

Hargrave, B. T., and G. A. Phillips. 1981. Annual in situ carbon dioxide and oxygen flux across a subtidal marine sediment. Estuar. Coast. Shelf Sci. 12: 725-737.

Howarth, R. W. 1979. Pyrite: its rapid formation in a salt marsh and its importance in ecosystem metabolism. Science 203: 49-51.

Howarth, R. W., and J. M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceanogr. 24: 999-1013.

Howarth, R. W., and J. M. Teal. 1980. Energy flow in a salt marsh ecosystem: the role of reduced inorganic sulfur compounds. Amer. Nat. 116: 862-872.

Howes, B., R. Howarth, J. Teal, and I. Valiela. 1981. Oxidation-reduction potentials in a salt marsh: spatial patterns and interactions with primary production. Limnol. Oceanogr. 26: 350-360.

Joint, I. R. 1978. Microbial production of an estuarine mudflat. Est. and Coastal Mar. Sci. 7: 185-195.

Jorgensen, B. B. 1977a. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. 22: 814-832.

Jorgensen, B. B. 1977b. Distribution of colorless sulfur bacteria (Beggiatoa spp.) in a coastal marine sediment. Mar. Biol. 41: 19-28.

Jorgensen, B. B. 1977c. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Mar. Biol. 41: 7-17.

Jorgensen, B. B., T. Fenchel. 1974. The sulfur cycle of a marine sediment model system. Mar. Biol. 24: 189-201.

Jorgensen, B. B., J. G. Kuenen, and Y. Cohen. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol. Oceanogr. 24: 799-822.

Keister, D. L. 1978. Respiration vs. photosynthesis, pp. 849-856 in R. C. Clayton and W. R. Sistrom (eds.) The Photosynthetic Bacteria. Plenum Press.

Kellog, W. W., et al. 1972. The sulfur cycle. Science 175: 587-596.

Kepkay, P. E., R. C. Cooke, and J. A. Novitsky. 1979. Microbial autotrophy: a primary source of organic carbon in marine sediments. Science 204: 68-69.

Kepkay, P. E., and J. A. Novitsky. 1980. Microbial control of organic carbon in marine sediments: coupled chemoautotrophy and heterotrophy. Mar. Biol. 55: 261-266.

Kleiber, P., and T. H. Blackburn. 1978. Model of biological and diffusional processes involving hydrogen sulfide in a marine microcosm. Oikos 31: 280-283.

Krasilnikova, E. N., Z. V. Pedan, and E. N. Kondrateva. 1976. Growth of purple sulfur bacteria in dark under anaerobic conditions. Microbiology 46: 503-507.

Lovelock, J. E., R. J. Maggs, and R. A. Rassmussen. 1972. Atmospheric dimethyl sulfide and the natural sulfur cycle. Nature 237: 452-453.

Lovelock, J. E. 1974. CS2 and the natural sulfur cycle. Nature 248: 625-626.

Madigan, M. T., and T. D. Brock. 1976. Quantitative estimation of bacteriochlorophyll c in the presence of chlorophyll a in aquatic environments. Limnol. Oceanogr. 21: 462-467.

Marker, A. F. H. 1972. The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshwater Biol. 2: 261-385.

Martens, C. S., and R. A. Berner. 1972. Interstitial water chemistry of anoxic Long Island Sound and sediments. I. Dissolved gasses. Limnol. Oceanogr. 22: 10-25.

Matheron, R., and R. Baulaigue. 1972. Bactéries photosynthètiques sulfureuses marines. Archiv. Mikrobiol. 86: 291-304.

Mendelssohn, I. A., K. L. McKee, and W. H. Patrick. 1981. Oxygen deficiency in Spartina alterniflora roots: metabolic adaptation to anoxia. Science 214: 439-441.

Novitsky, J. A., I. R. Scott, and P. E. Kepkay. 1981. Effects of iron, sulfur, and microbial activity on aerobic to anaerobic transitions in marine sediments. Geomicrobiol. J. 2: 211-223.

Oviatt, C., B. Buckley, and S. Nixon. 1981. Annual phytoplankton metabolism in Narragansett Bay calculated from survey field measurements and microcosm observations. Estuaries 4: 167-175.

Pamatmat, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. Int. Revue Ges. Hydrobiol. 53: 211-298.

Parkin, T. B., and T. D. Brock. 1980. Photosynthetic bacterial production in lakes: the effect of light intensity. Limnol. Oceanogr. 25: 711-718.

Peck Jr., H. D. 1974. The evolutionary significance of inorganic sulfur metabolism. Soc. Gen. Microbiol. Symp. 24: 241-262.

Peterson, B. J., R. W. Howarth, F. Lipschulz, and D. Ashendorf. 1980. Salt marsh detritus: an alternative interpretation of stable carbon isotope ratios and the fate of Spartina alterniflora. Oikos 34: 173-177.

Pfennig, N. 1978. General physiology and ecology of photosynthetic bacteria, pp. 3-18 in R. C. Clayton and W. R. Sistrom (eds.) The Photosynthetic Bacteria. Plenum Press.

Pfennig, N. 1978. General physiology and ecology of photosynthetic bacteria, pp. 3-18 in R. C. Clayton and W. R. Sistrom (eds.) The Photosynthetic Bacteria. Plenum Press.

Pfennig, N., and H. Biebl. 1976. Desulfuromonas acetoxidans gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch. Mikrobiol. 110: 3-12.

Pfennig, N., and H. G. Trüper. 1981. Isolation of members of the families Chromatiaceae and Chlorobiaceae, pp. 279-289 in M. P. Starr (ed.) The Prokaryotes. Springer-Verlag.

Postgate, J. R. 1969. Sulfate reducing bacteria, pp. 611-628 in J. R. Norris and D. W. Ribbons (eds.) Methods in Microbiology Vol. 1. Academic Press.

Postgate, J. R. 1979. The Sulfate Reducing Bacteria. Cambridge University Press.

Powell, E., M. Crenshaw, and R. M. Rieger. 1979. Adaptations to sulfide in the meiofauna of the sulfide system. I. S-35 accumulation and the presence of a sulfide detoxification system. J. Exp. Mar. Biol. Ecol. 37: 57-76.

Ramm, A. E. 1972. Some aspects of the sulfur cycle in tidal flat areas, and their impact of estuarine water quality. Thesis, Oregon State University.

Ramm, A. E., and D. A. Bella. 1974. Sulfide production in anaerobic microcosms. Limnol. Oceanogr. 19: 110-118.

Reise, K., and P. Ax. 1979. A meiofaunal 'Thiobios' limited to the anaerobic sulfide system of marine sand does not exist. Mar. Biol. 54: 225-237.

Revsbech, N. P., B. B. Jorgensen, and O. Brix. 1981. Primary production of microalgae in sediments measured by oxygen microprofile, C-14 fixation, and oxygen exchange methods. Limnol. Oceanogr. 26: 717-730.

Riemann, B. 1978. Carotenoid interference in the spectrophotometric determination of chlorophyll degredation products from natural populations of phytoplankton. Limnol. Oceanogr. 23: 1059-1066.

Smith, J. H. C., and A. Benitez. 1955. Chlorophylls: analysis in plant materials. pp. 142-196 in K. Peach and M. Tracey (eds.) Moderne Methoden der Pflanzenanalyse, Vol. 4. Springer.

Sorensen, J., B. B. Jorgensen, and N. P. Revsbech. 1979. A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. Microb. Ecol. 5: 105-115.

Strickland, J. D. H., and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Fisheries Board of Canada Bulletin 167.

Talling, J. F., and D. Driver. 1961. Some problems in the estimation of chlorophyll a in phytoplankton, pp. 142-146 in Proc. Conf. Primary Production Measurement Marine Freshwater, Univ. Hawaii U.S. A.E.C. Publication TID 7633.

Trüper, H. 1970. Culture and isolation of phototrophic sulfur bacteria from the marine environment. Helgo. Wiss. Meeresunters. 20: 6-16.

Trüper, H. 1978. Sulfur metabolism, pp. 677-690 in R. C. Clayton and W. R. Sistrom (eds.) The Photosynthetic Bacteria. Plenum Press.

Tunnicliffe, V., and M. J. Risk. 1977. Relationships between the bivalve Macoma balthica and bacteria in intertidal sediments: Minas Basin, Bay of Fundy. J. Mar. Res. 35: 499-507.

Tuttle, J. H., and H. W. Jannasch. 1977. Thiosulfate stimulation of microbial dark assimilation of carbon dioxide in shallow marine waters. Microb. Ecol. 4: 9-25.

Uffen, R. L. 1978. Fermentative metabolism and growth of photosynthetic bacteria, pp. 857-872 in R. C. Clayton and W. R. Sistrom (eds.), The Photosynthetic Bacteria. Plenum Press.

Uffen, R. L., and R. S. Wolfe. 1970. Anaerobic growth of purple nonsulfur bacteria under dark conditions. J. Bacteriol. 104: 462-472.

Van Baalen, C. 1961. Studies on marine blue-green algae. Bot. Mar. 4: 129-139.

van Gemerdan, H. 1974. Coexistance of organisms competing for the same substrate: an example among the purple sulfur bacteria. Microb. Ecol. 1: 104-119.

Van Raalte, C., W. C. Stewart, I. Valiela, and E. J. Carpenter. 1974. A C-14 technique for measuring agal productivity in salt marsh muds. Bot. Mar. 17: 186-188.

Van Raalte, C., I. Valiela, and J. M. Teal. 1976. Production of epibenthic salt marsh algae: light and nutrient limitations. Limnol. Oceanogr. 21: 862-872.

Vosjan, J. H. 1975. Respiration and fermentation of the sulfate-reducing bacterium Desulfovibrio desulfuricans in a continuous culture. Plant and Soil 43: 141-153.

Waterbury, J. B., and R. Y. Stanier. 1981. Isolation and growth of cyanobacteria from marine and hypersaline environments, pp. 221-223 in M. P. Starr (ed.) The Prokaryotes. Springer-Verlag.

Widdel, F., and N. Pfennig. 1977. A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, Desulfotomaculum (emend.) acetoxidans. Archiv. Mikrobiol. 112: 119-122.

Zinder, S. H., and T. D. Brock. 1978. Dimethyl sulphoxide reduction by microorganisms. J. Gen. Microbiol. 105: 335-342.

TYPED BY: Carolyn Sherrell