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STRUCTURE AND DYNAMICS
OF THE MARINE ANAEROBIC MICROBENTHOS

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Nearly all ecosystems of land and water including the open sea show a vertical zonation of physical and chemical factors and of flora and fauna. In such transitory zones there frequently exists interfaces where air meets land or light meets darkness or one physical condition gives way to another. Such an interface occurs within the sands and sediments of sea basins. The oxygenated surface layers of marine sand bottoms and beaches give way in deeper layers to anaerobic-reducing conditions with the exception of surf swept high energy beaches. Such interfaces frequently have biological significance.

The extent of the anaerobic-reducing biome is indicated by the size of the ocean basins themselves excluding areas without sediment and the "high energy windows" of surf swept beaches. In comparison, the anaerobic biome far exceeds the size of the aerobic surface sediments, although a great deal more scientific work has been done in oxygenated sediments.

Bacteriologists and geologists, (Zobell and coworkers, 1938-1948), probed and characterized the anaerobic sediments while researching the strata as a possible site of petroleum and sulphur formation. Investigations of the biota of deeper anaerobic layers have been all but completely ignored by biologists although the oxygenated layers of lake and marine

sands and the anaerobic lake and river sediments have been the subject of considerable research (Fenchel, 1969).

"Marine biologists traditionally have considered the "black zone" azoic as it contains toxic compounds such as hydrogen sulfide, and they have usually taken great care not to include H₂S-smelling sediments in their field samples." (Fenchel and Riedl, 1970).

Recent work in the biology and ecology of the anaerobic layers has been carried out and reported by the following scientists: Fenchel and Jansson, 1966; Fenchel, 1969; Riedl, 1969; Fenchel, 1970; Riedl and McMahan, 1970; Riedl and Ott, 1970. These studies were conducted in sands of Scandinavian waters and the East coast waters of the United States.

Within the scope of this essay, an attempt will be made to characterize and discuss the major physical factors and dynamics of the sulfide (anaerobic) layers. Special attention will be given to the biological significance of the redox-potential-discontinuity layer (RPD) where oxidizing processes commonly found in the upper sediments give way to reducing processes at the redox interface.

Physical Factors in Marine Beach & Bottom Sediments

Although the beach and bottom deposits are a variable and heterogenous mixture of sand, mud, water, detrius and biota, a general pattern exists. Variables in this pattern include sand particle size, organic content, pH, sediment layering, redox conditions, biota, and others which must be considered to appreciate the complexity of the marine anaero-

bic sediment ecosystem.

Methods do exist which rather precisely quantify the basic physical parameters of the system. However, the causal inner relationships of these physical and biological characters are extremely complex and are only now being elucidated. Consider the often studied relationship between particle size and biological interstitial inhabitants. Wieser (1959) established a correlation between particle size and distribution of specific organisms on five Puget Sound beaches. Some factors contributing directly to particle size were: current activity, sediment mother material, slope of beach, percent silt or clay, tidal flux, organic content, degree of sorting, biological activity, storms, etc. These particle size, influencing factors bring about subtle variations in the interstitial environment which are considered to be of biological significance.

In general, the anaerobic marine sediment biome exists in all bottom sediments and on gentle intertidal slopes where sand or mud conditions prevail and mean grain size is small enough to provide high water holding capillarity. Such beaches do not drain down significantly when tidal water recedes, thus a constant anaerobiosis prevails. Within the particulate material of such beaches and subtidal sediments, oxygen levels are extremely low and reducing conditions exist. One might expect that water currents of high oxygen content flowing over such sediments would introduce laminar flow and oxygenate the sands to considerable depth. Studies by Fenchel ('69) determined a 50% reduction of flow at the water-sediment

interface. In sands screened to 500 microns no H_2O movement was detectable below .5 cm. Thus in medium and fine sand, laminar flow does not appear to be of great importance.

Sands are said to be either well sorted or mixed. In well sorted sediments forces which move sand are such that particles of a given size are more or less found together in either a vertical or horizontal gradation. Such sands exhibit porosity characteristics typical of their mean grain size. Porosity is a measurement of intraparticulate space which in submerged strata is occupied with water. Such well sorted substrates typically show a porosity between 25-40% by volume, depending on the particle shape as well as grain size. In general, the better sorted the sand, the higher the porosity. In poorly sorted sand, the mixture of small and larger particles results in a filling in of interstitial spaces by geometric complementation which results in a reduction of porosity.

Permeability, the rate of flow through sediments, is closely related to porosity. It is of greatest importance on beaches which exhibit laminar flow as a result of wave action or tide activity draining down the beach slope through the sand. Permeability is most specifically related to sediment properties and can be reduced by a factor of four in well sorted sand by the addition of 2.5% silt (Webb, 1958).

Capillarity forces are determined by substrate composition, particle size, sorting and molecular interaction between the water and particle surfaces (adhesion). The closer together the particles and smaller the particle size the

greater the capillarity forces. Capillarity is especially important in considerations of beach drainage. High capillarity reduces laminar flow and promotes anaerobic conditions. Lentic conditions which are typical of backwaters, bays, sounds, subtidal and estuarine sediments promote low porosity and high capillarity because of their more typical fine texture sediments.

The anaerobic biome is world wide in distribution and is much more extensive than the more frequently studied oxygenated layers. Anaerobic sediment conditions extend from high water to beyond the continental slope, except in areas of "high energy windows". These "windows" are defined as those beaches which intercept 20 to 200 metric tons of clean seawater per beachfront meter per day (Riedl, 1969). Such beaches could be termed surf stressed. Beaches which show significant water turbulence, porosity and slope contain high oxygen levels during periods of high laminar flow but quickly revert to conditions of low oxygen levels when overlaid by stable water (Gordon, 1960).

As one goes seaward, such intertidal oxygenated sands rapidly change to benthic anaerobic layers.

It was found by Zobell (1943) that the addition of pure sand to marine bacterial cultures influenced their population numbers. He theorized that surface area was related to the availability of organic compounds. Marine water contains minute amounts of organic materials in solution and the availability of these compounds appears to be enhanced by increasing the surface area on which these materials may

accumulate. Hence the magnitude of interstitial surface has been indicated as important to the interstitial biome. The surface area for a 20 cc core in sand whose mean particle diameter is 300 μ is about 0.1 square meter. In general, the smaller the sand, the greater¹⁵ the surface area.

Oxygenated surface layers almost without exception rest on an anerobic foundation. Thus within the vertical layers of almost any sandy beach or marine basin, a sulfide or anaerobic biome will be found. Such strata may be located within one mm of the surface to 1 or more meters deep. It is characterized by pH and Eh (oxidation-reduction potential) readings which can be examined in situ using core probes designed by Riedl and Ott (1970, correr description in preparation). The pH of oceanic water ranges between 8.1 and 8.3 while within the sediments pH values are interrelated with temperature and E_h , and are typical for each type of sediment. Eh is measured in millivolts and is an expression of a system's oxidative or reducing tendency. It may be defined as the electron escaping tendency of an oxidation-reduction system. Thus the Eh value is an intensity factor. Eh values range in sediments from +350 to -500 mV., becoming more reducing with depth and increasing pH value. Plus Eh values are generally characteristic of bottom deposits which are well oxygenated, coarse and organically poor. Negative Eh values indicate layers rich in organic matter, consisting of fine sediments. The Eh capacity, defined as the resistance of a sediment to Eh change, first decreases with depth

then stabilizes at the RPD layer then continues to decrease in deeper sediments. The general pattern of Eh in marine sediments thus shows a higher resistance to Eh change at the RPD layer. Units of Eh capacity are known as poise and are defined according to colorimetric titrations against milliequivalents of methylene blue reduced per gram sediment (Zobell, 1946).

The H Ion and Eh nature of sediments are major controls which determine the kinds of chemical end members within the sediment system. The environmental consequences are biologically significant (Krumbein and Garrels, 1952).

Vertical Zonation

The boundary of the anaerobic biome is located where oxygen input is not sufficient to keep pace with organic deposition and subsequent decomposition. Thus the physical nature of the sediments largely determines the depth of the anaerobic or reducing layers. Typically the vertical distribution of sediments which include anaerobic zones can be described as made up of three layers: a top yellow layer, a middle gray layer (RPD), and a black layer. Within the top layer, oxygen is abundant, decreasing with depth. Eh values in the yellow top layer range between a high of +350 to +400 mV. to about +300 mV. decreasing with depth. The yellow layer derives its color from the presence of ferric iron.

It is in the gray zone that reducing conditions and oxidative conditions merge. Oxygen is present in most lim-

ited amounts and reducing compounds such as H_2S are present in small amounts. Eh values are diagnostic of these conditions as their indices quickly decrease to negative values. This layer, the gray zone, constitutes a newly discovered interface biome. Fenchel and Riedl call it the redox-potential-discontinuity (RPD) layer. It is the zone of oxidative-reductive confluence. The center of the RPD is defined as the flattest part of the Eh curve and its thickness as one-half the total change of Eh within a core. The Eh values of the RPD typically range from +300 mV. to negative values within the gray (RPD) zone. (See figure 1) Its organic content is typically between 0.01% and 0.8% with clay

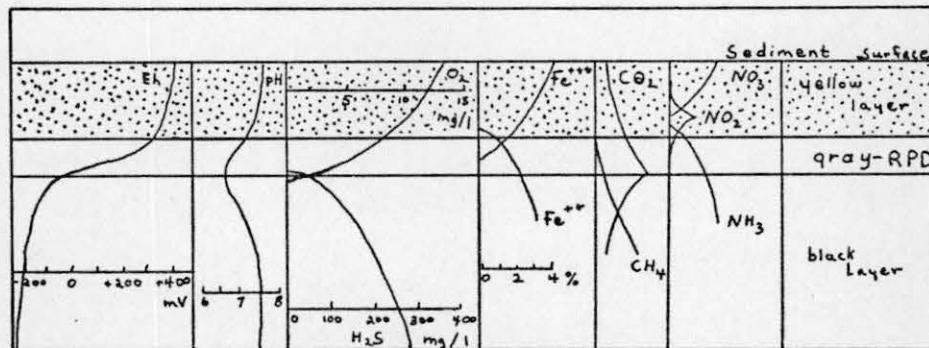


Fig. 1. Schematic representation of Eh and pH-profiles and vertical distribution of some compounds and ions in the vicinity of the RPD layer. Fully oxidized layer dotted (from Fenchel, 1969).

values between 0.5% and 5% depending largely on the amount of water turbulence or "protection".

Beneath the RPD gray layer is the "black zone", or "sulfide zone". Oxygen levels are non-existent while H_2S occurs in large amounts. Eh values for the black sulfide zone are between -100 and -500 mV. (Zobell, 1946). The black coloration of this layer is due to the presence of iron sulfides. Conditions in this layer are strongly reducing.

The marine sediments do not always exhibit the color bands as listed, but they are characteristic of quartz sands, muds, and clays. Calcareous sands typical of tropical regions exhibit the physiochemical zonation but colors are poorly developed. Thus, with exceptions in "high energy windows", the three layers with their characteristic conditions exist in most marine sediments.

Changes in water currents, porosity, beach slope, permeability, sorting, silting, etc. may cause the upper sediment layers to compress. The RPD layer may be as little as millimeters beneath bottom sediments in lentic (quiet) beaches and sea bottoms or meters below the surface in conditions which result in high laminar flow as on steep lotic beaches. The lower limit of the black layer is defined by termination of bacterial activity and often typified by accumulation of poisonous compounds, disappearance of organic matter, by cementation and by recrystallization of the newly formed products of mineralization.

RPD Vertical Migration

The sediments are not to be thought of as static in conditions or depth location. Water movements which bring about greater protection of marine deposits, as in sand spit building, causes the RPD to rise toward the surface. Seasonal changes which result in high temperatures also cause the RPD level to rise as rates of organic decomposition lowers oxygen penetration reducing the oxygenated surface sediments. Winter storms which may expend vast energy on mixing sand

layers increase oxygenation and drive the RPD layers deeper. However, frequently such storm activity increases the deposition of organic drift material which forces the RPD upward toward the sediment surface. Photosynthetic activity within the upper cm introduces a circadian influence which, under conditions favoring a near surface RPD, results in a temporary daytime depression of the reducing layers during periods of oxygen production.

Such minimal displacement, however, is quickly offset by oxygen uptake during periods of murky water or darkness. Thus the RPD layer is not static but is influenced by biological activities and non biological processes (Gordon, 1960; Brafield, 1964, 1965; Fenchel, 1969).

Dynamics of the Sulfide Layers

The complex chemical and biochemical dynamics of the sediment layers are the product of a one way system of light, oxygen, and organic matter availability. Light penetration into the sediment layers is limited even in ideal sediments to approximately three cm. Organisms do exist which have a low compensation point enabling some productivity within the sediments, i.e. benthic diatoms (Taylor, 1964).

Experiments were conducted by Fenchel, 1969 to determine the extent of sediment productivity and oxygen balance for sediments in situ. Ten liter aquaria were inverted and forced ten cm into water-covered sediments forming a chamber enclosing sea water and undisturbed sediments. Oxygen balance within the chamber was monitored under ideal light con-

ditions in shallow water. He found in all cases that the oxygen production of photosynthesis did not replace the oxygen requirements for the enclosed system. This experiment further indicates that the bottom-sediment heterotrophic ecosystem is based on imported organic matter; oxygen being supplied primarily by diffusion from the surrounding watery environment.

Aerobic organisms which occupy the surface layers quickly deplete oxygen levels. Since organic input from deposition of both micro and macro detritus exceeds the available oxygen supply for aerobic breakdown in the sediments, most of the energy yielding biochemical reactions are anaerobic. Organisms do exist within the sediments which can convert the complex organic substances into inorganic molecules with no biologically useful energy content.

The conversion of these energy rich molecules to biological use involves several steps of degradation by organisms especially adapted in utilizing end products of previous organisms. Thus, general patterns of distribution within the sediments occur as each organism modifies its environment, providing conditions and materials acceptable to another trophic level. Since sediments are most frequently subtended by a zone of sand-rock diagenesis, barriers exist which prevent downward escape of accumulating metabolic end products. Concentration gradients occur which cause net transport of these end products toward the surface where they are important in secondary productivity in the presence of oxygen and sometimes light.

Thus, general cyclic progression of biological activity occurs due to the one way input and surface escape of transient products of the marine sediment layers (Fenchel, 1969). (See figure 2)

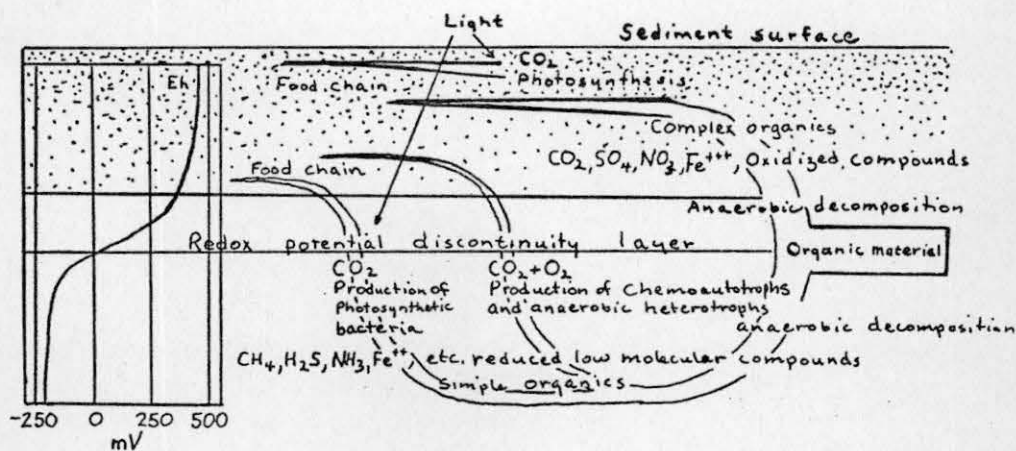


Fig. 2. Schematic representation of energy flow and some major microbial processes. Vertical section through the sediment in the vicinity of the RPD layer. Oxidized area dotted. (After Fenchel, 1969; modified)

In attempting a more detailed synthesis of the sediment cycles, the general color zonation of Fenchel and Riedl, '70 will be followed. It must be realized that the many physical parameters previously discussed will influence the compression or expansion and even the existence of these zones in any given sediment complex. For example, in extreme lentic conditions, the anaerobic layers may extend several meters above the sediment surface into the overlying stable water precluding the existence of the yellow layer in such sediments (Mortimer, 1942; Baas, Becking et al., 1957). More frequently, the yellow and gray layers may be compressed to less than .5 mm thickness with essentially all included metabolic processes being anaerobic (Fenchel, 1969).

In marine sediments where the yellow (ferric) layer is well developed, organic input results in oxidative metabolism by micro-organisms. Oxygen concentration is greatest at the sediment surface where photosynthesis in sediments and diffusion from adjacent oxygen-containing water provides a source of supply. Oxygen in the yellow layer is rapidly consumed, decreasing in concentration as a function of depth due to oxidative respiration and non biological oxidation of reduced substances. Heterotrophic metabolism is controlled by a diminishing supply of hydrogen acceptors as oxygen levels decrease. The presence of partially digested organic compounds and scarcity of oxygen provides conditions suitable for fermentation by bacteria and fungi in which other organic materials act as hydrogen acceptors. This fermentation results in further reducing conditions and more CO_2 production.

Changes in Eh and pH reflect the changing metabolic activity. Surface Eh values of approximately +350 drop to +200 mV. within the yellow layer. As hydrogen ion concentration increases, pH drops from levels of approximately pH 8 at the sea substrate interface to pH 7 at the RPD. Below the RPD, pH values increase to approximately 9.5 with depth. NO_3 concentration rapidly changes to more reduced forms of Nitrogen, NO_2 and NH_3 . The ferrous ion (Fe^{+++}) indicative of the yellow layer gradually changes to the gray ferric (Fe^{++}) ion as biological activity continues building reducing conditions. Oxygen concentration at the bottom of a typical yellow zone is approximately .7 mg/1 of interstitial water.

Toward the lower limits of the gray layer, oxygen concentration falls to near zero level as reflected in Eh values which descend from +200 to zero. The Redox interface surrounding the zero Eh level is the previously defined Redox Potential Discontinuity (RPD) layer. Fermentation reaches its maximum at lower limits of the gray layer in the RPD zone. This fermentation results in maximum CO₂ production within the sediments and the production of large amounts of organic acids, alcohols, etc. Further fermentation is limited by the lack of hydrogen acceptors which influence thermodynamic efficiency.

The Eh curve (See figure 2) indicates a poisoning effect within the gray layer which is primarily attributed to the electron absorbing capacity of the Fe⁺⁺⁺ → Fe⁺⁺ system. Thus, these common ions in this system may be thought of as poisoning agents which influence the rate of Eh change within the sediments (Fenchel, 1969; Fenchel and Riedl, 1970).

Below the RPD boundary the black sulfide (Iron sulfide) layers exist. The color of the black layer is caused by Desulphovibrio sp. bacteria which oxidize H₂ by reducing SO₄ to H₂S (Baas, Becking and Wood, 1955). This H₂S may then be converted to black iron sulfides; Eh levels below +100 mV. favor this sulfate conversion. So central is sulfide concentration to Eh values in this zone that these values can be calculated directly from sulfide concentration data (Zobell, '46, '48). Eh values below -250 mV. are not reported by Fenchel (1970).

Biological activities within the black layer are restricted to anaerobic heterotrophs and other bacteria which break down organic products of fermentation. These organisms utilize inorganic compounds for H acceptors. Thus, NO_2 is converted to NH_3 , CO_2 to CH_4 , Fe^{+++} to Fe^{++} and SO_4 to H_2S making these products accumulate with H_2 in the black layer.

As these materials diffuse upward from the lower anaerobic black sediments where they were formed, Eh values become positive within the RPD layer. This change in redox enables chemautotrophic bacteria to synthesize organic compounds from CO_2 , using the reduced inorganic compounds as both a source of hydrogen and energy.

Therefore, end products of anaerobic respiration are linked to the new production of organic matter. These autotrophic reactions tend to promote higher Eh (more oxidative) conditions which support the physical conditions of the upper sediments. Thus, chemoautotrophic activity results in converting NH_3 to NO_3 , CH_4 to CO_2 , H_2S to SO_4 , etc. which are then recycled as hydrogen acceptors for decomposers of the black layer. These simple metabolites act as energy carriers from the anaerobic to the aerobic zones within marine sediments.

In sediments where the RPD layer is close to the surface, light and inorganic reduced metabolic products being available, photoautotrophic organisms such as Oscillatoria, Pinnularia, and white, purple, and green sulfur bacteria carry out photoreduction of CO_2 . Such organisms utilize

light for energy and, also, take advantage of reduced inorganic compounds for an oxidizable hydrogen donor.

Reduced inorganic substances which do not first encounter chemoautotrophs or photoautotrophs are spontaneously oxidized by free oxygen if encountered, reverting to their oxidized form. Such oxidation results in energy loss to the sediment ecosystem (Fenchel, 1969).

The work of Sorokin (1965, '69) in stagnant lakes focused the attention of marine biologists not only upon the trophic importance of anaerobic decomposition but upon the biological utilization of resulting reduced end products. The production which results is comparable to primary production because the energy source cannot be utilized by higher organisms directly. However, in reality, this available energy is the result of previous photosynthesis.

It is of additional interest that the RPD appears to be the site of maximum energy availability to sediment organisms as evidenced by maximum biomass in the vicinity of the RPD layer.

Convergence of materials and conditions which contribute directly to the biological importance of the RPD are summarized as follows:

1. The CO_2 cycle within the sediments provides maximum reducible CO_2 at the RPD layer.
2. Inorganic metabolites of the black layer diffuse upward carrying energy to the RPD layer.
3. Environment conditions, brought about by oxygen diffusion and autotrophic activity at the RPD, maintain Eh

conditions which allow organisms to take advantage of available energy.

4. Since metabolic end products of autotrophic activities do not accumulate at the RPD layer, an environment persists which does not limit this activity.
5. Highest sediment fermentation rates center on the RPD. This provides CO_2 levels for the autotrophs which supply organic products for anaerobic activities. Further reduction results in the inorganic metabolites which furnish energy for secondary productivity.
6. Aphotic chemoautotrophs occur abundantly throughout the earth's RPD and under photic conditions are assisted by photoautotrophs. Autotrophs carry on CO_2 reduction resulting in energy rich organic compounds. These materials recycle into the black layer.

In the "Volga Reservoirs Study" Sorokin found bacterial biomass and bacterial biosynthesis to be maximal in the RPD layer. This bacteria population supports a large population of animals which feed on bacteria (Fenchel and Riedl, 1970). Within the final section of this paper, an attempt will be made to summarize the present understanding of the marine biological community dependent upon this energy source.

Biology of the Oxidation - Reduction (RPD) Layer

In his 1957 summary of marine ecology, Hedgpeth includes a chapter by Gunnar Thorson which discusses bottom communities. His discussion deals primarily with the epifauna, but enough reference is made to the infauna to gather

a level of understanding for the period. Quoting Thorson, "The marine level bottom ecologist is in a much more favorable position; he can use the large uniform bottom flats and the "parallel communities" for well-founded generalizations, but he must not forget the responsibility which this privilege gives him in finding ways to fill the gap from marine to terrestrial ecology."

The extensive insights provided by Fenchel, 1969; Wieser and J. Kanwisher, 1961; Riedl, 1969; and Hansen, 1965 into the marine microbenthos destroys the myth of a simple ecosystem within the sediments. Fenchel's 191 page treatise which emphasizes the ecology of marine ciliates establishes clearly the specific geographical and vertical organization within the sediments and their lack of homogeneity wishfully predicted by Thorson.

Geographical regions have been well documented in the study of catenulids (Turbellaria) and gnathostomulids. Not more than one of the sixty gnathostomulid species found on Western Atlantic shores is identical to the twenty species found in Europe. Only one species of the gnathostomulid group has been reported from both sides of the Atlantic and from the Pacific (Kirsteuer, 1964).

The exacting analysis of ciliate distribution discussed in Fenchel '69, presents a clear case for specie specific vertical zonation. In his Alsgarde sediment sample twenty-three organisms or groups of organisms are tabulated, each showing discrete depth distribution dependent upon physical and biological factors. Eh levels and H₂S concentration are

thought to be largely directive in establishing vertical distribution within the sediments.

The classical bacteriological and physical descriptions of sediments by C. E. Zobell (1938-1948) lead the way for more detailed biological work. Much descriptive work has been done in marine sediments, but ecological work not only demands identification of species but most laborious quantification of the ecosystem in situ.

The most successful research in this endeavor typically involves measurement of pH and Eh followed by core extraction, sectioning the core into vertical intervals, extraction of organisms from each interval and finally microscopic examination of extracted organisms for species determination and digestive system content. A systematic analysis of sand grains is done for identification of the nonextractable forms.

Research into the microbenthos communities of the seas is still at best a pioneering activity. Major work has been done in European waters and on the Western Atlantic coast by Riedl, et al. Examination of the sediments is laborious work. Fenchel's research in Danish waters used the sea water ice method of Uhlig (1964, 1966) which has been shown to give extraction efficiencies of 70 to 90% for marine ciliates (Fenchel, 1967) and 75% for ciliates, flagellates, turbellarians (Uhlig, 1964). The methods are considered good for gastrotrichs and archiannelids while other groups of nematodes, rotifers, harpacticoids and tardigrades are less well extracted. Fenchel found a 60% efficiency for nematodes. It must be concluded that data gathered by the Uhlig method is sys-

tematically 10-20% too low compared to absolute count.

Penetration of organisms from other biotopes occurs mainly from the overlying oxidized layers.

At Helsingor Beach, Denmark, where the oxidative upper layers are well developed, data on diatom distribution has been determined. It was found that populations of 20 diatoms per 50 sand grains at the photic surface continued to 14 cm levels which were aphotic, reducing, having Eh of zero. These and other transient organisms appear to be facultative anaerobes and aerobes whose rate of reproduction and longevity within the reduced layers continues to be largely undetermined. However, the net effect of such invasion into less desirable living conditions is an increased localization of potential energy in deeper sediments (Fenchel, 1969).

There are, however, species which prefer the oxidative-reductive interface, the RPD. These belong to four of the five kingdoms advanced by Whittaker (1969): Monera, Protista, Fungi and Animalia (metazoa).

Major Monera (procaryotic organisms) present in the RPD layer are the Cyanophyta, Eubacteria, and the spirochaeta.

Dozens of species of blue-green-algae are represented throughout the sediment system in aphotic zones. In the Helsingor sediments, blue-green-algae were found below 10 cm depth in an Eh of -100 mV.

The blue-green-algae are mostly representatives of Oscillatoria which become especially dense when the RPD layer approaches the surface and light is available. However, in the Helsingor Beach samples, populations of 200 filaments per 0.01

cc were recorded at 8 cm depth. The highest populations of Oscillatoria terebriformis were found within the RPD layer at $2\frac{1}{2}$ cm from the sediment surface where counts of 1200 filaments per 0.01 cc sediment were recorded in an Eh of +20 mV. Oscillatoria margantiferae occurs only to depths of 1 cm at Helsingor Beach and is thought to be more light dependent.

It is of interest that blue-green-algae in these sediments show so little light dependence. The average population through twelve cm of sediment was 400 filaments per 0.01 cc sediment but a maximum of 12,000 filaments per 0.01 cc was found in the RPD. It is seen, therefore, that large populations of blue-greens occur throughout the aphotic sulfide layers and appear to be largely heterotrophic.

The blue-green component, unlike most other interstitial organisms, increases in population and diversity when grain size, sorting, porosity and permeability decrease; and as the RPD moves closest to the surface (Fenchel and Riedl, 1970; Fenchel, 1969).

Eubacteria and Spirochaeta play a most important role within the RPD. They provide links between aerobic photosynthesizers and anaerobic heterotrophs.

Most general bacteriological references which deal with the marine biome refer back to the 1930's and the work of C. E. Zobell and associates. It is of special interest that his work included not only descriptive analysis of bacterial forms but also critical analysis of physical data.

Zobell (1938) divides marine sediment bacteria into

three classes: Aerobic, Anaerobic, and Faculative anaerobic. In his analysis of bottom sediments he concluded that neither depth nor distance from the mainland seemed to have any influence on Bacteria of bottom deposits. His samples ranged from coastal waters to 120 mi at sea and to 2,009 meters deep. Zobell's data was taken off the coast of southern California.

Plating procedures were used to determine bacterial counts from core samples, and Eh - pH and substrate conditions were dutifully recorded. He found in deep sea samples that the oxidative layer, though hard to determine, occupied the upper 1 mm to 5 mm of the sediments. Bacterial counts for anaerobic and aerobic bacteria were recorded as follows from samples taken off Channel Island, California.

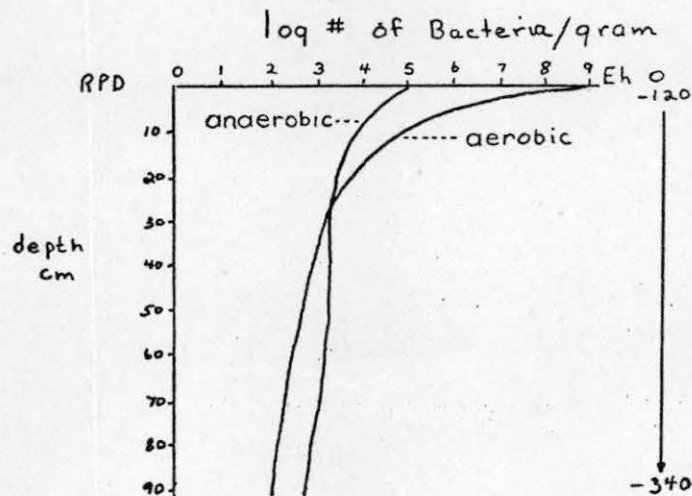


Fig. 3.

Zobell concluded that aerobic bacteria below 3 cm depth were slowly dying as they settled into the sediments and more reducing conditions. His graph does indicate a rapid decrease in Aerobic bacteria; however, since faculative bacteria would

test aerobic by aerobic plating methods, it is suspected that many of the deep aerobes found were equally able to carry out normal life activities by anaerobic means. Counts of anaerobic bacteria were interpreted as dying below 50 cm where populations were $\approx 10^3$ /g. sediment.

Low oxidation - reduction potential retards decomposition of organic matter and prolongs the viability of bacteria which multiply slowly under these conditions. At this point one wonders what becomes of bacteria which continue downward, escaping the diminishing chance of being biologically consumed. I have seen no data on this thought but conclude there must be an organic residue protected by deep lying, extremely reducing conditions.

Zobell found bacteria were more numerous at the sea sediment interface, which is also the RPD in these sediments. Smaller particle size sediments produced highest bacteria counts. Temperatures for sediment bacteria were found to be optimal at 20° C although deep sea sediment conditions of 5° C simply reduced multiplication rate. These bacteria can multiply at 0° C. It was further found that bacteria from deep in the sediment layers were not haline sensitive and could live as well in fresh water mud.

Data taken by Zobell off Channel Island, California, indicates $\approx 10^{12}$ bacteria per gram sediment within the RPD layer (Zobell, 1938, 1946; Zobell and D. O. Anderson, 1936).

The Bacterial findings of Zobell in California waters are consistent with the general details reported by Fenchel (1969), from Denmark. Although Fenchel's major interest was

the ecology of the ciliates, his details of the trophic ecology of the sediments support the findings of Zobell.

It is of further interest that Fenchel tested his general sediment stratification findings and ecological patterns of distribution in models he constructed in his laboratory. His models were of several designs and only the simplest will be detailed here (see Fenchel, 1969).

Models were constructed by placing a small crystal of Na_2S in a glass tube of sea water. This sulfur compound served as an energy source for sulfur bacteria. The tube was sealed at one end and closed at the other by a cotton plug. The system was inoculated by addition of a few drops of water from a natural sample of estuarine sand. He found in some cases the supply of sulfide was too high, killing all organisms in the tube. Other tubes grew successfully following a phase of succession in which growth of *Thiobacillus* and filamentous sulfur bacteria became established. The growth was located some distance from the end of the tube, antecedent of ciliate populations. Slightly more complex model systems adopted from Baas Becking (1925) were inoculated with drops from Niva Bay, Denmark. Within 24 hours, the Eh of the lower parts of these tubes dropped drastically due to bacterial activity. The Eh, over several days, fell to values around -200 mV. A fluctuation of Eh followed when reduced compounds from the sulfide zone began to recycle into the upper tube layers causing the redox discontinuity layer to move downward.

At the level of the RPD layer, ± 0 mV., dense masses of

various kinds of bacteria occur. Filamentous sulphur bacteria, mainly Beggiatoa which appear after 2-5 days, persist as long as H_2S is formed. Other white sulfur bacteria and bacteria not belonging to the sulfur cycle also occur. Different kinds of bacteria develop at different levels in the tube forming bacterial plates or horizontal zones. Above the redox discontinuity layer the number of bacteria decrease, Protozoa, Amoebae, and ciliates developed in abundance, and zonation by Eh was detected. The experiment just discussed was conducted under dark conditions and the results observed remained rather stable until energy sources collapsed, terminating the community after several months.

Similar model communities kept in the light began similar development with bacterial plate formation composed of Beggiatoa and other colorless bacteria with related fauna development. In the following days, however, green and purple sulphur bacteria and other photosynthetic bacteria became increasingly abundant, first at the RPD and later extending downward. The green bacteria always occurred below the purple ones. Diatoms were present in large numbers and were eventually found distributed throughout the culture. The photosynthetic organisms became more abundant at the expense of the nonphotosynthetic bacteria. Blue-green algae Oscillatoria became common and, after one month, were quite dominating.

Since photoautotrophic organisms, in contrast to chemototrophs, can remove reduced compounds under anaerobic conditions, their activity destroys the original zonation of

the chemical factors and, subsequently, the zonation of the flora and fauna.

It is significant that the model systems of Tom Fenchel closely exemplify through succession those conditions which exist in natural sediments. The bacterial distributions of Zobell and Fenchel's own finding of the RPD system is strongly supported by these simple models.

Major principles supported *in vitro*:

1. Maximum biomass distribution occurs at the oxidation reduction interface.
2. Recycling of deep reduced inorganic products of metabolism function as energy and electron sources for CO₂ reduction by chemoautotrophic bacteria.
3. The importance of darkness was illustrated in the system for the establishment of physical-chemical-biological stratifications typical of the bottom sediments.
4. Under oxidative conditions no typical physical-biological zonation develops as in high energy beaches.

Before continuing the discussion of the relative involvement of different animal groups within the sediment layers and their relationship to the RPD, it should be mentioned that the total fauna, comprising all sizes of animals, has probably only a modest share in the metabolic activity of the sea bottom when compared to bacteria. The use of detritus by animals has been found largely dependent upon bacterial activity (Zobell, 1963; Wieser and Kanwisher, 1961; and Carey, 1967).

Thus, the significance of bacterial distribution is apparent as the bacterial role in energy cycles is elucidated. Most frequently the energy of detritus cycles through bacteria to other consumers within the sediments.

Protista

The protista include organisms which are of eucaryotic organization. The reducing layers contain a large number of phyla. Autotrophs, such as euglenoids, diatoms and dinoflagellates, are observed in great numbers in the upper reducing layers. Vertical migrations of these forms is thought to occur, but the ecological role of this migration is not yet understood.

Studies of diatom distribution in the sediments were conducted by Fenchel on Julebaek Beach, Helsingor Beach and from sediments of Niva Bay. In general, viable diatoms were found to a maximum depth of 16 cm in the sediments. Until recently it was assumed that diatoms only occur at or close to the surface, but evidence is in hand which determines that diatoms penetrate well into the sediments. No tide affected the beaches studied and periods of calm water persisted prior to Fenchel's sampling so a mechanical mixing of surface sediments by water turbulence cannot account for the diatom distribution. Well established Eh zonation, also, constituted evidence that the sediments were undisturbed.

Sediment samples from Helsingor Beach in March and May showed diatom populations to average 8 and 38 diatoms per fifty grains of sand respectively. These values were taken

at a depth of 12 cm and illustrated that diatoms are not just surface phenomena. Much higher diatom population levels were found at these depths in Niva Bay but unfortunately, population density in that study was designated in arbitrary units of relative abundance. One can only say from the data that populations at 12 cm depth were one-fourth the maximum near surface populations in the Bay samples.

Because of the photosynthesis capabilities of diatoms, one would expect maximum population counts at the sediment surface. This was frequently the case as in the Julebaek samples. Counts at the surface were four times the counts at the 3 cm level. Eh values ranged from +450 mV. at the surface to +375 mV. at 3 cm and a well developed Eh stratification, also, existed to -150 mV. with depth. The Niva Bay sediments exhibited a well developed Eh stratification from +450 mV. to -150 mV. at 13 cm depth. However, maximum populations of diatoms were found at the RPD located between one and four centimeters deep. In the Helsingor Beach sediments in which the RPD layer was deeper 6 to 9 cm, a combination of the previous conditions of maximum populations was detected.

Subsurface populations were highest at 1-4 cm but this was above the RPD; while within the RPD diatom populations were about 90% of the subsurface (possibly light influenced) maximum. It can only be concluded from Fenchel's 1969 data that diatom populations are influenced favorably by light as expected and, also, by conditions of the oxidation-reduction-discontinuity (RPD). Both light influenced and RPD zones were sites of maximum diatom habitation.

Because of their chlorophyll content, it was previously

assumed that diatoms found in aphotic layers had slipped or migrated downward from the photic zone. Good evidence does exist in support of diatom migration in response or anticipation of tide activity (Callame and Debyser, 1970); however, work by Lewin (1953, 1963) has shown that a number of pennate and centric diatoms can live extended periods in aphotic conditions by heterotrophic means. Thus, there is a controversy involving diatom nutrition and mode of life within the sediment layers. Studies of diatom species distribution within the sediments must be determined, followed by study of species requirements within simple model laboratory systems to resolve the controversy. Because an organism contains chlorophyll one cannot conclude that it is restricted to active photosynthesis.

Diatoms are important within the sediments for their primary production capabilities, apparent distribution of food to lower sediment layers, and their role as a major source of food to other organisms, especially ciliates.

Among the flagellates important within the reducing sediments are the Euglenoids and the Dinoflagellates. Dinoflagellate populations have been determined and are in the order of 1000 to 2000 individuals per square cm or 2g/square meter to depths analyzed.

In fenchel's study of the ecology of the marine microbenthos, highest concentrations of Gymnodinium were found in the sulfur contaminated sediments. These populations were often in excess of 6000 Gymnodinia per cm², frequently associated with purple sulfur bacteria in Niva Bay and also

abundant in Julebaek Beach where 1400 per cm^2 have been counted. Gymnodinium appeared to be photosynthetic and was found close to the surface.

In clean sediments, species of Amphidinium were found to dominate. Many of them were devoid of chloroplasts and occurred at considerable depths. Flagellate populations were seen to fluctuate greatly and vertical migrations were strongly indicated.

Euglenoids were most common in sulfur dominated sediments where interstitial water contained 40 mg H_2S per liter.

Distributions of Dinoflagellates appear more related to the availability of light than to Eh. However, in the Alsgarde sediments where maximum populations were found at 6 to 8 cm, Eh conditions were representative of the RPD.

The extent and ecological importance of the vertical migrations has not been determined. Dinoflagellates and other flagellated protista have proven to be a major food source contributing energy to the ciliate populations studied by Fenchel (1969).

As previously indicated, heterotrophs are very important in the sediments. Phyla largely heterotrophic among the protista are Sarcodina, Ciliphora and Zoomastgophora. Among these only the ciliates have been studied in great detail within the sediments. They are represented by a few families but over a dozen genera whose population densities of 1000 to 30,000 individuals per 100 cm^2 are found in this system.

Fenchel (1969) is a major contribution to understanding

both anaerobic and aerobic sediment systems. The work was carried out mainly in European waters where 83 quantitative samples were taken from a broad range of sediment types, some of which have been cited in this paper. Major attention was given by Fenchel to ciliate forms. These were carefully counted by species using best known techniques to preserve both numerical data and physical relationships within the sediments. Non-ciliate Protista, metazoa and other organisms were counted in less specific groupings by phyla. Included in his lengthy paper are charts of specie distribution and ecological relationships including food preferences for over eighty-four species or generic groups of sediment ciliates. A number of important generalizations concerning sediment ecology can be gathered from his work.

Ciliates, like other organisms within the sediments, have their own distinct distributions. These are often quite species specific, showing optimum Eh levels with populations for each specie, decreasing both above and below optimum conditions.

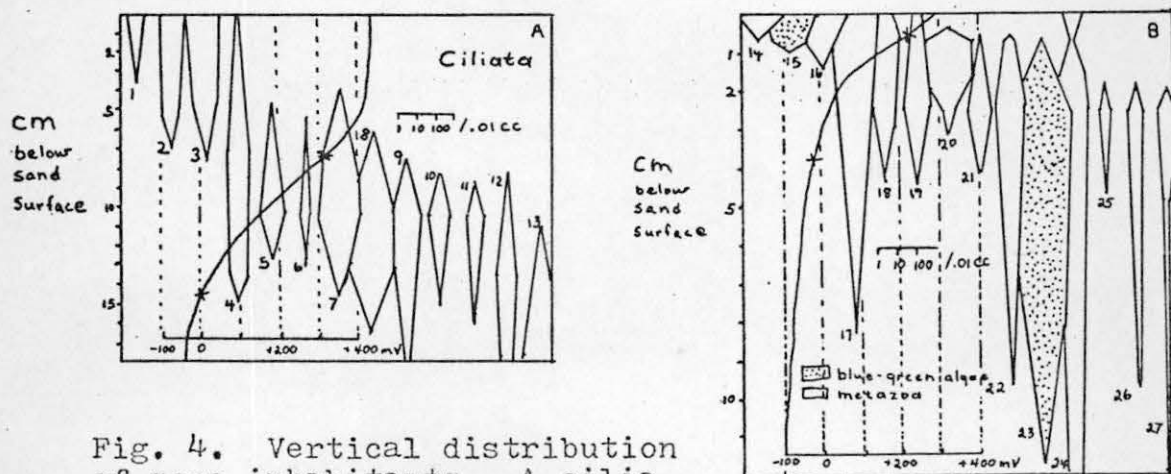


Fig. 4. Vertical distribution of some inhabitants. A ciliates from Helsingor Beach, Denmark, May; B blue-green-algae

and metazoans from Wrightsville (McCrary's) mudflats, North Carolina, October. 1 Loxophyllum sp., 2 Frontonia arenaria, 3 Pleuronema coronatum, 4 Lacrymaria sp., 5 Remanella brunnea, 6 Paraspatidium fuscum, 7 Remanella margaritifera, 8 Mesodinium pupula, 9 Sonderia cyclostoma, 10 Remanella minuta, 11 Metopus contortus, 12 Caenomorpha levandri, 13 Mesodinium sp., 14 Arthropoda (harpacticoids, ostracods, halacarids), 15 Oscillatoria margantiferae (about 3 species) 16 Metachromadora onyxoides (nematodes), 17 Gastrotricha (2 species not yet described), 18 Semaegognathia sterreri (gnathostomulid), 19 Acoela (turbellarian, 4 species not yet described), 20 Terschellingia longicaudata (nematodes), 21 Gnathostomula microstyla (gnathostomulid), 22 Oscillatoria terebriformis, 24 Eubostrichus parasitiferus (nematode), 25 new marine Catenulida (turbellarians, 3 species), 26 Gomphonema typica (nematode), 27 Filiospermoidea (3 species gnathostomulid), (A- original by Fenchel, 1969; B- original by Riedl)

Fenchel found nine genera of ciliates which contain one or more species that can live completely in anaerobic environments for long periods. During the Fenchel 1969 study, forty-one samples (cores) from five sites were analyzed for H₂S and species present. The contents of H₂S varied from 0 to 700 mg per liter. It can be concluded from this that different species of ciliates in sediments show different ranges of tolerance to oxygen and H₂S. The vertical distribution of the animals may be controlled by the distribution of these compounds which partially explains the correlation between vertical distribution and Redox profile. Many of the ciliates endure or require high concentrations of H₂S.

Anaerobic decomposers will be found in the strongly reducing layers of the sediment and it seems probable that the majority of inhabitants of the sulfide zone feed on these bacteria.

As previously pointed out, maximal bacterial biomass in most sediments examined by Fenchel is found within the RPD layer. It was further determined at five major sites that 46% of the total ciliate food spectrum is composed of bacteria while 35% is composed of diatoms and flagellates. The values also varied due to seasonal fluctuations when studied in Helsingor Beach sediments from October through August 1966-67.

Within this study, major ciliates' foods were diatoms, flagellates and bacteria. When bacteria decreased as a major food source for the ciliates, the use of the diatom-flagellate resource showed a compensational increase. Graphs of data from Helsingor Beach indicate that greater than 85% of ciliate food was composed of bacteria, flagellates and diatoms.

Of the 34 species or genera divisions tallied at Helsingor Beach, the following table shows the distribution of ciliate fauna related to depth and Eh.

depth cm.	population per segment	Eh mV.	
0-3	91	+454	
3-6	61	+466	
6-9	169	+442	
9-12	526	+220	} RPD
12-15	218	+154	
15-18	130	-026	

Table 1.

Fungi

Fungi are the least studied group within the sediments. However, hyphae of the lower fungi are frequently found in the gut of gnathostomulids from the sulfide layer. Such evidence indicates that saprophytic marine fungi do occur in oceanic sediments. Indeed, all the lower phyla of true fungi (Plasmodiophoromycota, Hyphochytridiomycota, Oomycota and Chytridomycota) may be expected to be present (Kohlmeyer, 1966; Riedl, 1969).

Metazoa

The metazoa in the sulfide biome mainly include the two most conservative bilaterian phyla, the Platyhelminthes and Aschelminthes. The Platyhelminthes are represented by both their non-parasitic classes, the Turbellaria and Gnathostomulida. The Aschelminthes are mainly represented in the sulfide biome by classes Nematoda, Gastrotricha, Rotatoria and Oligochaeta.

Although numerous workers have examined these organisms in the sediments, most interest has been in their identity and distribution as related to sediment median grain size. Few studies include Eh stratification which allows an analysis of animal distribution and RPD. Only the most superficial analysis of the metazoa and their Eh distribution is justified at this time.

Studies by R. J. Riedl of the Wrightsville mudflats of North Carolina point up representative metazoan distribution within the sulfide biome. (See figure 4B) He found Nematodes

to be most deeply distributed within the sulfide system. Eubostrichus parasitiferus ranged from the sediment surface through the total depth of the sample which was 12 cm. Other nematode species showed more specialized distribution. Meta-chromadora onyxoides, for example, was not found below 1.3 cm beneath the surface. Nematode populations ranged from 300 to 9 per 1/100 cc of sediment. Maximum counts for subsurface (below 1 cm) species were found in the RPD layer. These nematode findings are typical for the metazoans. Species are distributed in the Eh strata according to their own requirements; thus, there are species which must live in more porous oxidative sediments and those which can tolerate more reducing conditions. In general, the metazoa prefer more oxidized environments but there are species which do prefer sulfide sediments.

Seven turbellarian species, six gnathostomulid species and two gastrotrich species were extracted by Riedl, counted and distributions determined in the Wrightsville mudflat samples. (fig. 4B) He found the metazoan species to have limited distributions with their major populations in the RPD. The sediments were fine, conditions mildly to severely anaerobic and population counts ranged from 4 to 300 individuals per 1/100 cc sediment.

Fenchel and Jansson (1966) studied the vertical distribution of microfauna in the sediments at Askö in the Baltic Sea. Sediments were not as fine as at Wrightsville, having a median grain diameter of 430 μ at the surface to 230 μ at the 2-3 cm depth which was the location of the RPD. Nematoda,

harpacticoids, rotifers, oligochaets, chironomida larvae and turbellarian groups were almost entirely located above the RPD in the more porous, less reducing sediments. Less than ten percent of these metazoans were found distributed downward to a depth of 6-10 cm where the Eh was -200 mV. Greatest species diversity was found in the surface sediments. Populations were low among the metazoans in these sediments with total counts per cm^2 of 57 to 99. Approximately 25% of these were nematodes.

Recent papers discussing the distribution of gastrotrichs in Florida beaches, rotifers in Scandinavia, and turbellaria in Niva Bay indicate a modest role but high trophic level within the food chain for this group of organisms.

Most species of micrometazoans decrease rapidly in population as reducing conditions of lower sediments are encountered. Diatoms, dinoflagellates, blue-green algae and bacteria constitute the major food source for the common metazoans. (Thane-Fenchel, 1968, '70; Straarup, 1970)

Sulfide System Toxicity

It is obvious that those organisms restricted to the sulfide biome have physiological adaptations which enable them to survive lack of oxygen and toxic conditions. That the sulfide biome is temporary quarters for organisms which have slipped or "dived" into the RPD and sublayers becomes more and more unlikely in view of mounting evidence. Noland and Gojdics (1967) examined 14 sulfide inhabiting species of ciliates enduring more than 300 mg H_2S / liter of inter-

stitial water; nine have been cultured anaerobically with H_2S levels of 42 to 295 mg/liter.

The toxicity of NH_3 and H_2S is related to the pH conditions of the sediments. Low pH levels promote lower toxicity but sediment pH levels seldom go below pH 7 which occurs at the RPD and Eh of 0 mV. conditions. High NH_3 and H_2S concentrations are known to interfere with enzymes of the citric acid cycle and electron carrier cytochrome system. No ultra structure study has been done on invertebrates restricted to the sulfide biome. Two Gnathostomula genera have been examined and they do contain mitochondria, but they exist slightly above the RPD level. Further research is needed to determine the nature of the energy-transforming machinery of the sulfide restricted organisms.

Within the sulfide layers, some groups of organisms are conspicuous for their absence. Lacking are the higher fungi, Ascomycetes and Basidiomycetes, and, in addition, higher plants which are the eucaryotic multicellular autotrophs. These include all plants above and including green algae.

Among the animal groups not present in the sulfide layers are Arthropoda and many other metazoan phyla. Almost all Coelomates are missing from these layers. Further, many common types of aerobic sand fauna are absent: Turbellaria, Nemertini, Tardigrada, and Archiannelida. Missing also are dwarf types of Cnidaria, Echinodermata, Ascidiacea and others.

Summary

The anaerobic sediments comprise a complicated physical

and biochemical ecosystem which may prove to be the largest biome on earth. It is found underlying the earth's waters in all but coarse sediments overlaid with turbulent water. Thus, this biome is typical of oceans, lakes and estuary sediments.

Physical conditions are varied and often in a flux as affected by earth's natural cycles and random atmospheric events. Patterns within the anaerobic ecosystem are now being elucidated.

Sediment biologists have found an oxidation reduction (Eh) continuum within the sediments which has special biological significance. Within this continuum occurs a discontinuity layer where oxidative surface conditions become reducing. Populations of bacteria, blue-green-algae, diatoms and micrometazoan organisms have been found in greater density at this sediment discontinuity.

Explanations of this biological distribution anomaly have been sought and some theories have been advanced.

A measure of secondary productivity by chemoautotrophs is regarded as important in contributing to the trophic support of organism at the RPD. Such production is promoted by the physical gradients and material cycles which are biologically maintained within the sediments. Organic input occurs at the water-sediment surface, as does oxygen and light. Detritus thus introduced is acted upon by heterotrophic aerobes which remove oxygen and promote anaerobic conditions.

Available oxygen is not adequately abundant to allow complete aerobic breakdown, thus fermentation is promoted.

Products of fermentation encounter deeper layers which are strongly reducing where organic compounds, the biproducts of fermentation, are reduced by bacteria to inorganic electron acceptors. These inorganic molecules provide transport of energy and electrons upward to the RPD where, because of Eh changes, chemoautotrophic bacteria can gain thermodynamic purchase, reducing CO_2 to particulate organic matter. This organic matter contributes to the substantial biomass of the RPD layer.

The essential sediment stratification is an energy phenomenon built and maintained by biological activity in a confining environment. Experiments in model systems have shown darkness to be required for the building and continuence of the RPD and associated trophic relationships. If the RPD is near the surface, as in a sulfuretum, maximum production is at the surface as chemoautotrophs and photoautotrophs merge productivity.

The concentration of potential energy at the RPD initiates a flow of energy through trophic levels. Present evidence indicates that bacteria, diatoms and flagellates contribute their energy mainly to ciliate forms which are most numerous within the RPD.

Micrometazoa generally are found higher in the sediment complex. They appear to occupy a higher trophic position which may enable energy capture from the RPD without being confined to the layer.

Ciliate motility may function in this distribution.

Many micro metazoans require more oxidative environments; while some, like the nematodes, range through all layers.

One of the most interesting facets of this complex ecosystem is its apparent biochemical integration. Quantitative chemical research will likely elucidate this ecological complexity. Most basic is the need for a more complete interpretation of the measured electron potentials. Enumeration of all types of bacteria, especially the neglected anaerobic and chemoautotrophic forms from the sediments is essential. Dark fixation of CO_2 by chemoautotrophs is not adequately understood. Perhaps radio nucleids can be introduced to model sediments and biochemical techniques applied to detect energy flow patterns.

In recent years, some taxonomic groups have been well studied, others remain needing adequate investigation.

It is obvious that "simple" organisms constitute the populations of these reducing sediments. Perhaps relationships exist which are closely reminiscent of early life's seas and soups. It is hoped ecological relationships found here will be primary, disclosing fundamental principles of population dynamics and specie diversity.

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