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Distribution, abundance, and metabolic states of microorganisms in the water column and sediments of the Black Sea[1]

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Abstract

The vertical distribution, abundance, and metabolic states of microorganisms have been measured within the water column of the Black Sea. Samples collected from the O_2–HS^- interface revealed a vertically restricted layer (<20–30 m) of metabolically active cells. Within the anoxic portion of the water column (200–2,100 m), ATP concentrations were 5–10 times greater than in oxygenated environments of comparable depth; biomass and various metabolic activities increased with increasing water depth, implying a vertical gradient in specific organic substrates required for bacterial growth and metabolism.

The vertical distribution of ATP was also measured in two sediment cores raised from the western basin of the Black Sea. The ATP concentration within the uppermost sediment layer (0–10 cm) was 4 orders of magnitude greater than in the overlying water column, on a per volume basis (150 ng ATP·cm^{-3} for the sediments, vs. 10 ng ATP·liter^{-1} for the water column). Scanning electron micrographs are presented depicting early stages in the diagenesis of framboidal iron sulfide.

Within the past two decades extensive oceanographic investigations have been conducted in the Black Sea (RV *Vitjaz*, and RV *Lomonosov*, 1960; NOAA ship *Oceanographer* and RV *Pillsbury*, 1967; RV *Atlantis II*, 1969; RV *Chain*, 1975; and many cruises of the Institute of Oceanology, USSR Academy of Sciences). Although much information has been compiled concerning the role of microorganisms (Kriss 1959; Sorokin 1964, 1970; Tuttle and Jannasch 1973; Castellvi 1975), many basic ecological questions remain unresolved, or at best only partially so (*see* Jannasch et al. 1974). Of particular importance are the biological and chemical cycling of sulfur compounds, especially the relationships between the microbial sulfur cycle and the flux of organic carbon compounds within the water column and sediments (*see* Sen Gupta and Jannasch 1973).

Egunoff proposed in the early 1900s that the Black Sea must contain a "bacterial plate," a thin layer of sulfide-oxidizing bacteria that in his opinion served to prevent the diffusion of HS^- into the surface waters. This hypothesis was based primarily upon analogy with anisotropic lakes (Ravic-Scerbo 1930; Sorokin 1970). Using a laboratory model of the Black Sea, Ravic-Scerbo (1930) was able to produce an active layer of bacterial sulfide oxidation, adding experimental credibility to the Egunoff hypothesis. Since then, many workers have investigated the vertical distribution and metabolic activity of bacteria in the water column in order to assess the role of bacteria in biogeochemical transformations. Many of the published reports, however, are contradictory (e.g. Kriss 1959 vs. Sorokin 1964, 1970), in part perhaps due to problems inherent in the classical methods of counting bacteria.

Only a limited effort has been directed to the microbial populations within the sediments. Since bacteria can exert so profound an influence on sedimentary transformations (ZoBell 1946; Ivanov 1968; Sokolova and Karavaiko 1968; Zajic 1969), the diagensis of metal sulfides (especially iron sulfide) in anoxic environments has received a great deal of attention. The report by Degens and Ross

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(1974) has greatly expanded our knowledge of the geochemistry of recent Black Sea sediments and their interstitial waters.

In April 1975, estimates were made of the vertical distribution of microbial biomass within the water column and sediments by measuring adenosine triphosphate (ATP) and total adenine nucleotides (A_T) at several locations in the western basin of the Black Sea. From determinations of ATP, ADP, and AMP, the adenylate energy charge (EC) ratios

$$EC = \frac{ATP + \frac{1}{2}ADP}{ATP + ADP + AMP}$$

were calculated to establish the relative in situ metabolic state of the cells. The vertical distributions of particulate nucleic acids (PNA), particulate organic carbon (POC), and particulate organic nitrogen (PON) were also measured at one deep-water station. Samples were collected from the surface sediment layer (0–2 cm) and prepared for examination with the scanning electron microscope (SEM). I present data here on the distribution, abundance, and metabolic states of microorganisms in the anoxic waters and sediments of the Black Sea and discuss these results in terms of the availability of energy for bacterial growth and metabolism.

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Materials and methods

Sample collection—All samples were collected between 19 and 28 April 1975,

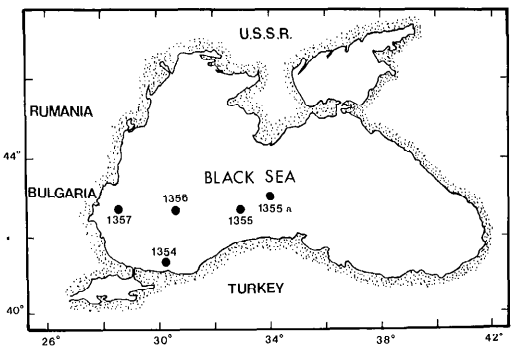


Fig. 1. Stations occupied during RV *Chain* cruise 120. Station locations: 1354—41°27′N, 30°14′E; 1355—42°50′N, 33°00′E; 1355a—42°59′N, 33°59′E; 1356—42°41′N, 30°37′E; 1357—42°38′N, 28°36′E.

at stations shown in Fig. 1. Water samples for ATP, A_T, HS^-, and O_2 determinations were collected with 30-liter Niskin bottles (General Oceanics), those for PNA, POC, and PON analyses with a 60-liter Bodman water sampler (Bodman et al. 1961). Sediment samples were taken at two stations by gravity corers and chemical and microbiological analyses begun immediately on board ship.

ATP, A_T, and EC: extraction, measurement and calculation—Water for ATP, A_T, and EC determinations was subsampled from the Niskin bottles by filtration through a 183-μ nitex screen into sterile Fernbach flasks. The adenine nucleotides (ATP, ADP, and AMP) were extracted according to the ATP method of Holm-Hansen (1973). The volume of water filtered varied between 100 ml and 1.5 liter, depending on the expected cell density of the sample. A shipboard ATP photometer (SAI Technol. Co.) was used to determine the optimum filtration volume for each depth interval, in order to minimize deviations from linearity (Sutcliffe et al. 1976; Karl unpublished). Whenever possible, duplicate water samples were extracted and analyzed; the error bars in certain figures represent the actual range of duplicate determinations. The water overlying each core was carefully siphoned off and the sediment extruded 1 cm at a time with a wooden plunger. Whenever the core was subsam-

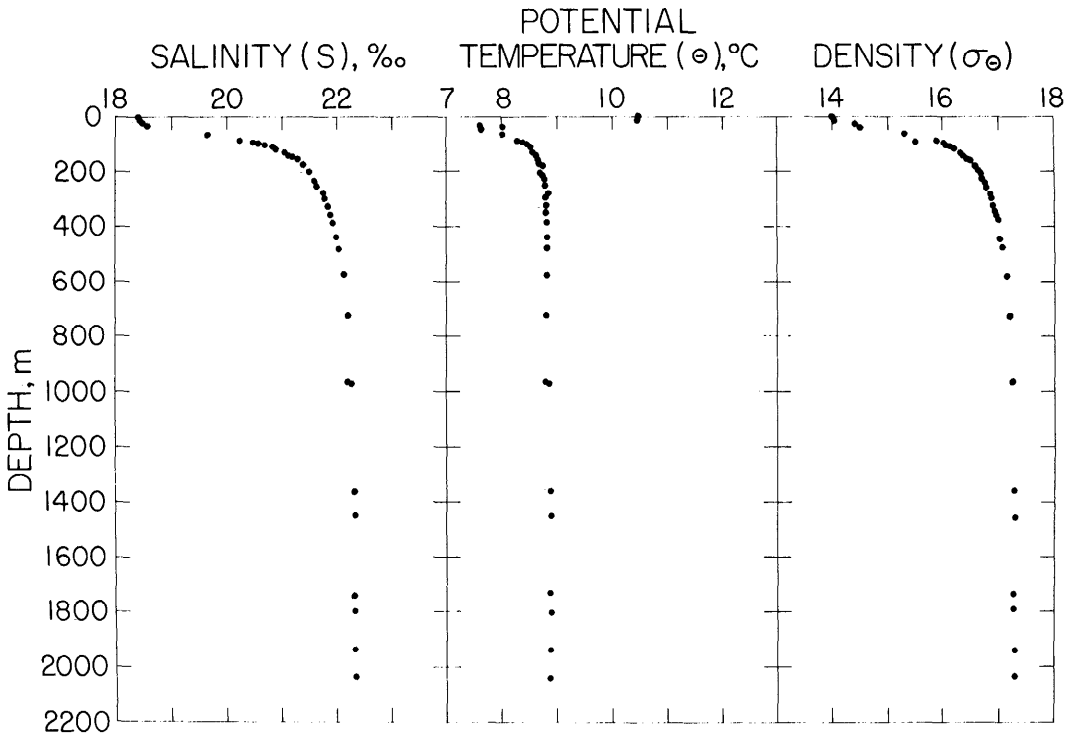


Fig. 2. Salinity, potential temperature, and density measurements for water column at station 1355.

pled, only the central portion was used; the material in direct contact with the core liner was discarded. ATP was extracted from triplicate subsamples by the sulfuric acid-EDTA procedure of Karl and LaRock (1975); the data shown for sediments represent the actual range of these ATP determinations. The nucleotide extracts were kept frozen (−20°C) for later analysis on land. ATP, A_T, and EC ratios were determined by a firefly bioluminescent assay procedure (Karl and Holm-Hansen in press), a modification adapted for environmental samples of the analysis described by Pradet (1967) and Chapman et al. (1971).

PNA—Particulate nucleic acids were extracted by prefiltering 5 liters of seawater through a 183-μ mesh and then concentrating the remaining material onto a microfine glass-fiber filter (Reeve Angel, type 984-H). The filters were extracted with cold (4°C) 5% (wt/vol) trichloroacetic acid (TCA), and the PNA concentration was measured spectrophotometrically by a colorimetric reaction (Schneider 1957). Since both DNA and RNA react with these reagents, the results are expressed as particulate nucleic acids (PNA).

A standard curve was prepared relating color intensity to known RNA concentrations. Purified yeast RNA (Sigma) (100 mg) was dissolved in 5 ml of 5% TCA by heating for 15 min. Various dilutions of this stock solution were prepared in 5% TCA to provide standards ranging from 0.2–20 μg RNA·ml^{-1} which were assayed as described above.

SEM—Surface sediment (0–2 cm) was subsampled as described above and prepared for SEM observation by a minor modification of the method of Paerl and Shimp (1973). After fixation, desalting, and dehydration, the samples were kept immersed in 75% ethanol (ETOH) until the critical point drying procedure which was performed on land. Although the samples underwent decompression before the initial fixation and were stored

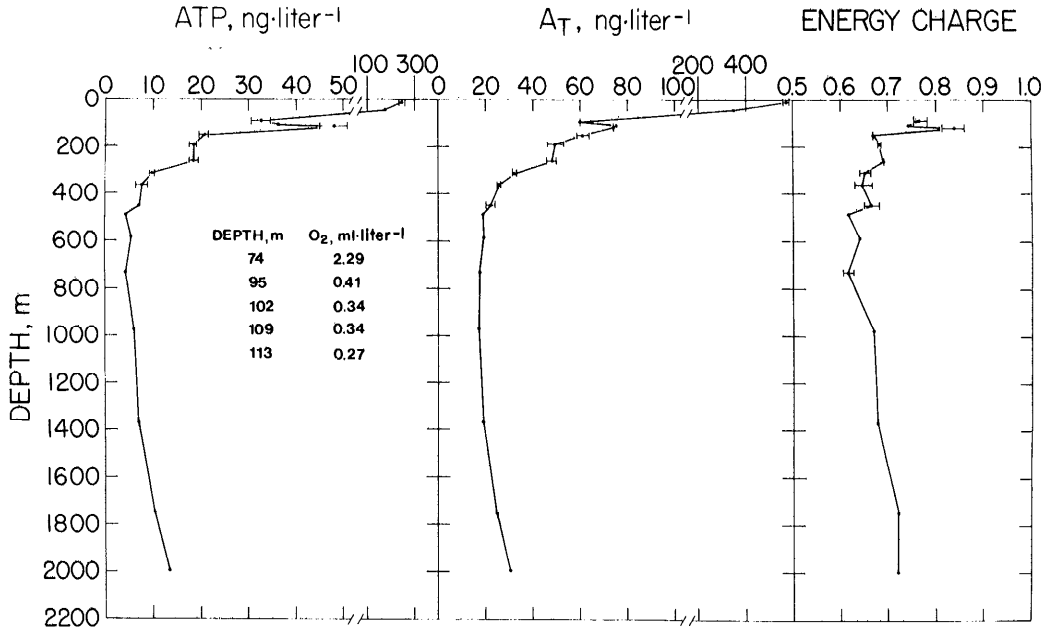


Fig. 3. Vertical distribution of ATP, A_T, and energy charge for water column at station 1355. Total water depth 2,160 m.

for a considerable time in 75% ETOH after the desalting and dehydration series, SEM observations show that neither procedure affects the cell morphologies (Carlucci et al. 1976; A. F. Carlucci pers. comm.). All samples were examined with a Cambridge stereoscan electron microscope and the images recorded on type 105 P/N Polaroid sheet film. The elemental composition of selected samples was qualitatively determined by energy dispersive spectrometry (EDS).

Hydrographic and chemical data—Measurements including potential temperature (Θ), salinity ($S‰$), potential density (expressed as $\sigma\Theta$), HS^-, O_2, POC, and PON were provided by R. B. Gagosian. Water samples used for these analyses were collected at stations shown in Fig. 1. The entire set of hydrographic and chemical data, together with a brief documentation of the methods used, is to be compiled into a single cruise volume (Gagosian in prep.).

Table 1. Vertical distribution of several important biological parameters within water column at station 1355.

Depth (m)	ATP ng·liter⁻¹	PNA μg·liter⁻¹	PNA/ATP	POC (μg·liter⁻¹)	PON (μg·liter⁻¹)	POC/PON	PNA-C* μg·liter⁻¹	PNA-C/POC
10	260	23.1	89	81	9.2	8.8	7.9	0.097
48	200	14.4	72	51	5.0	10.2	4.9	0.096
107	34.3	6.1	178	22	1.8	12.2	2.1	0.095
137	30.5	5.3	174	24	3.4	7.0	1.8	0.075
185	27	8.7	322	36	5.1	7.1	3.0	0.083
342	10.5	7.7	733	18	2.2	8.2	2.6	0.144
488	5.9	5.6	949	15	1.8	8.3	1.9	0.127
977	8.9	8.5	953	15	1.9	7.9	2.9	0.193
1,800	12	10.4	867	25	3.8	6.5	3.5	0.140
2,045	15	14.0	933	29	3.8	7.6	4.8	0.166

* Calculations based on average composition of yeast RNA.

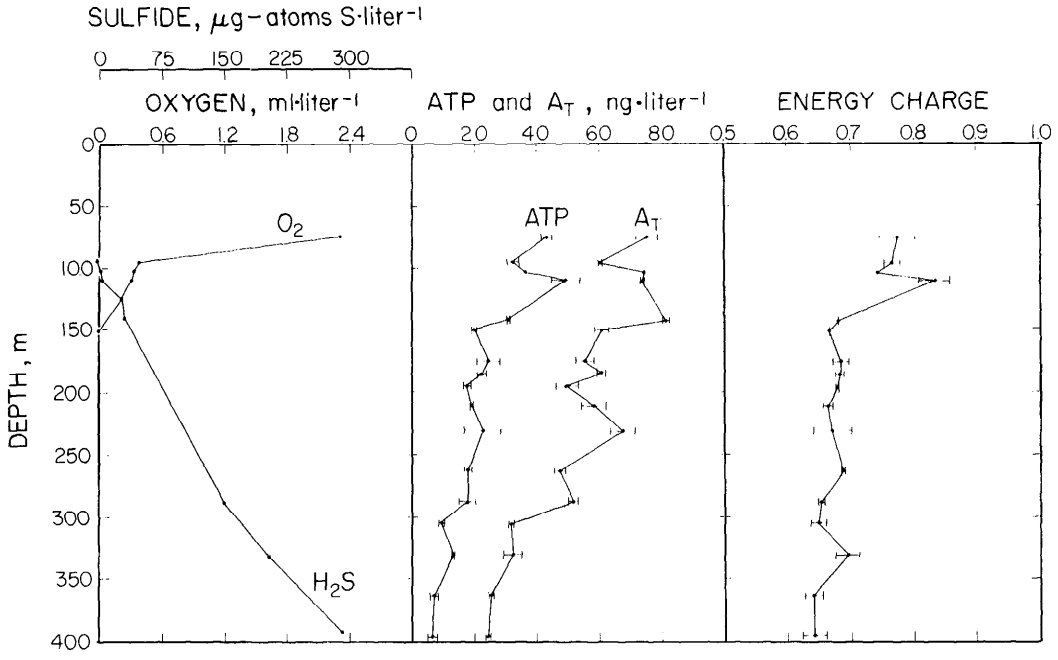


Fig. 4. Vertical distribution of HS^-, O_2, ATP, A_T, and energy charge for upper 400 m of water column at station 1355.

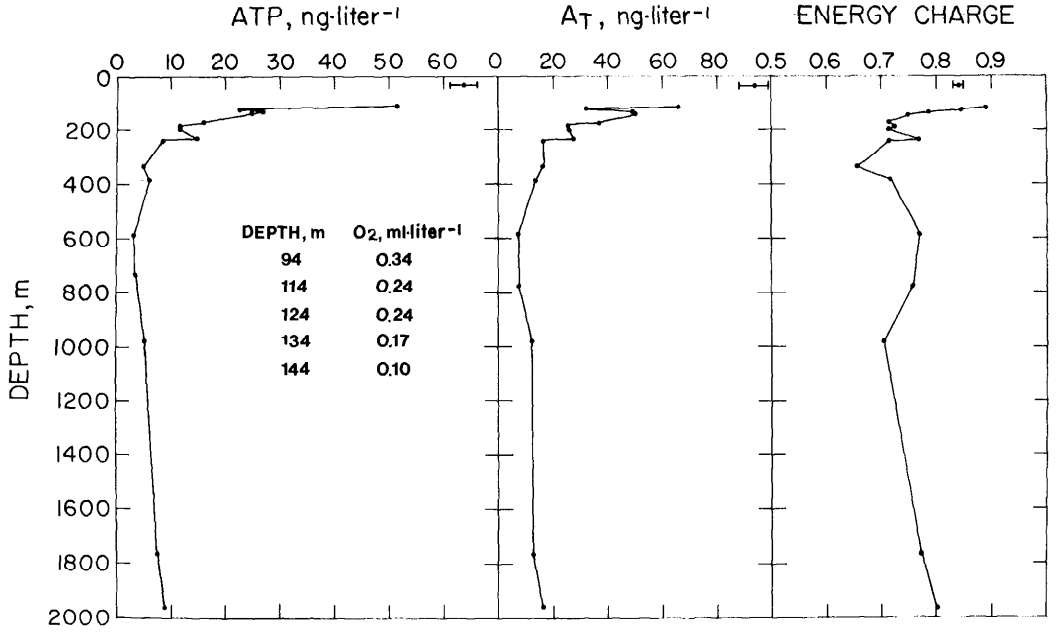


Fig. 5. Vertical distribution of ATP, A_T, and energy charge for water column at station 1356. Total water depth 2,180 m.

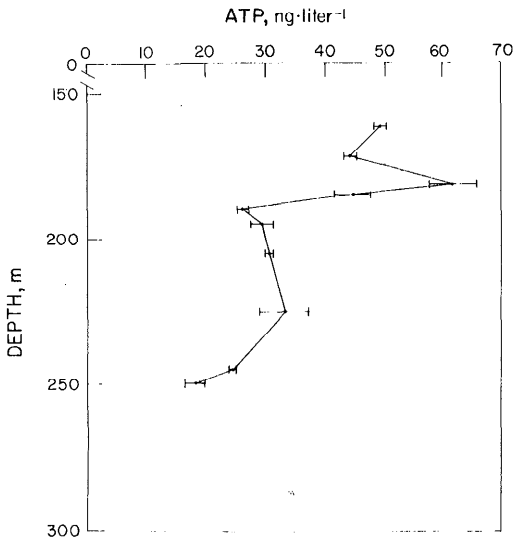


Fig. 6. Vertical distribution of ATP for water column at station 1357. Total water depth about 500 m.

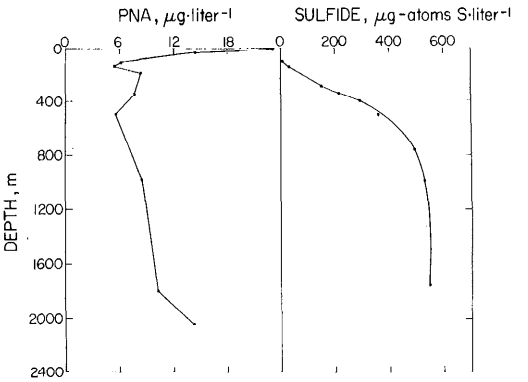


Fig. 7. Vertical distribution of PNA and HS^- for water column at station 1355. Total water depth 2,160 m.

Results

Figure 2 shows the vertical distribution of S‰, Θ, and σΘ for the entire water column at station 1355 (*see Fig. 1*). The most significant feature is the steep pycnocline between 50 and 150 m. This pycnocline, which is induced by rapid changes in salinity with depth, is the primary physical barrier to advective mixing and the ultimate cause of anoxic conditions in the Black Sea. Although hydrographic parameters were similar at all four hydrostations, only those data collected at station 1355 are presented here.

The results of the adenine nucleotide determinations (ATP, A_T, and EC ratios) revealed several interesting and consistent features. The vertical profiles were characterized by an increase in ATP and A_T in the region of the oxic–anoxic interface, accompanied by an increase in the EC ratio (Figs. 3, 4, 5, 6). The increase in ATP, A_T, and EC was located in the region where both O_2 and HS^- were present in low but detectable concentrations (Fig. 4). Directly below the subsurface peak, the A_T concentration remained relatively high for nearly 50 m, while the ATP and EC ratio peaks were of much more restricted vertical extent (Fig. 4). Below 800 m, the ATP and A_T concentrations increased with increasing water depth to deep water (>800 m) maxima at about 2,000 m (Figs. 3, 5), the deepest water sampled. This unusual increase in ATP with increasing water depth was accompanied by a concomitant increase in the EC parameter (Figs. 3, 5).

The vertical profiles of PNA, POC, and PON for station 1355 are presented in Fig. 7 and Table 1 and show general features similar to those of ATP, A_T, and EC. The subsurface peaks in PNA, POC, and PON were slightly deeper than those of ATP and EC, but were well within the region of the subsurface A_T maximum (Figs. 4, 7, and Table 1). Below 500 m, the distribution of PNA, POC, and PON also showed increasing vertical gradients.

The sediment ATP profiles were characterized by a subsurface maximum at a depth of about 3 to 6 cm (Fig. 8). The average ATP concentration in the top 10 cm of each core represents an increase of more than four orders of magnitude (on a per volume basis) over the concentrations in the deepest portion of the water column. Although the ATP surface (0–2 cm) and deep (10 cm) values were similar in the two cores, the concentration of ATP at the subsurface maximum was greater in the core from shallower water

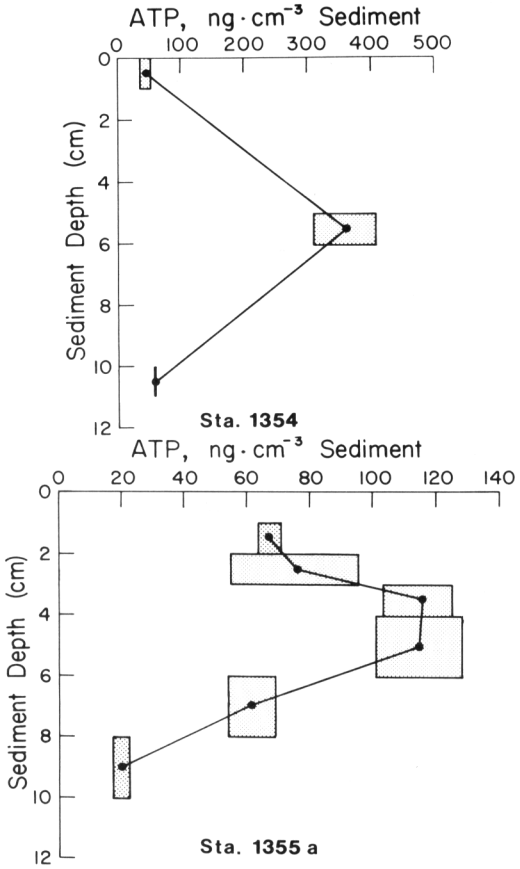


Fig. 8. Vertical distribution of ATP for sediments collected at stations 1354 and 1355a. Total water depth 450 m and 2,200 m.

(354 vs. 116 ng ATP·cm^{-3} wet sediment: Fig. 8).

Representative results of SEM observations from the sediment are presented in Figs. 9 and 10. The most conspicuous biogenic components of the deep-sea (2,200 m) sediment were coccoliths in various stages of dissolution and occasional diatom frustule fragments (Fig. 9A, B), consistent with previous observations (*see* Degens and Ross 1974). In addition to these calcareous and siliceous detrital fragments, there were many colonies of bacteria (Figs. 9C, 10A). Assemblages shown in Fig. 10 are considered to represent early stages in the diagenesis of iron sulfides and in the formation of pyrite framboids. Energy dispersive X-ray

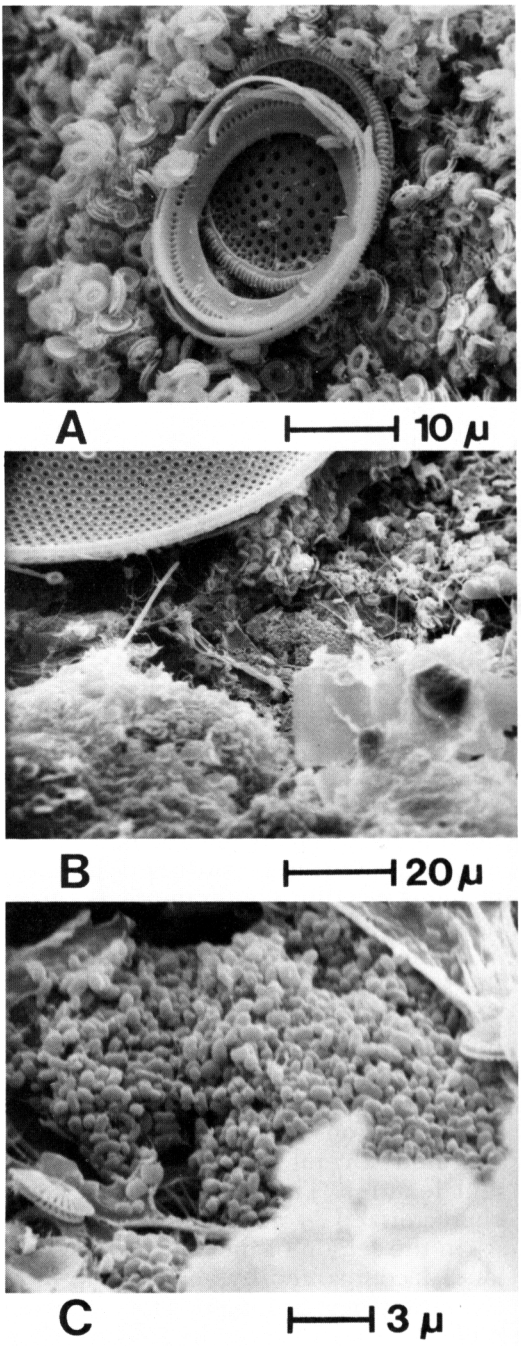


Fig. 9. Deep-sea (2,200 m) sediment environment and microenvironments. A—Indicates ubiquity of coccoliths and diatom frustules throughout topmost sediment layers. B—Habitat surrounding a bacterial colony (center right). C—Enlargement of colony shown in panel B.

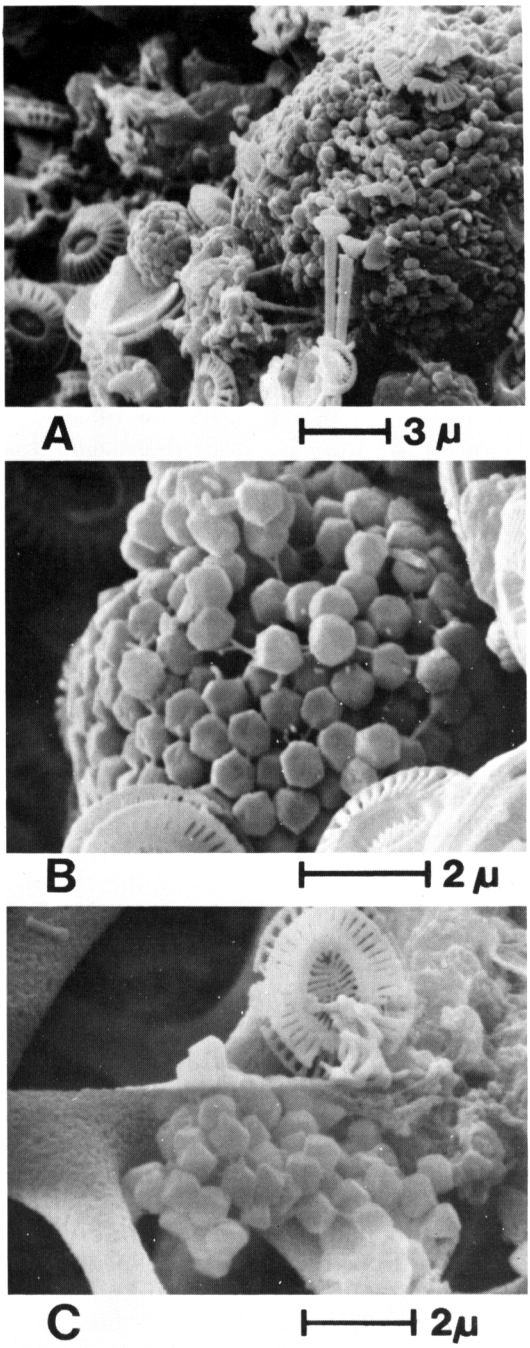


Fig. 10. Environments of iron sulfide precipitation. A—Iron sulfide framboid (left center) surrounded by dense bacterial growth (right side). Note similarity in size between bacterial cells (right) and individual microcrystallites of framboid. B—Framboid assemblage. Note mucuslike connections between individual components of framboid, suggesting biological origin. EDS analysis indicates that Fe and S are the two primary components of this framboid. C—Aggregate of individual framboid-producing particles with remains of various biogenic components (radiolarian, coccolith). Again, note mucuslike accretions connecting particles, but, most important, absence of characteristic spherical framboid shape.

emission spectra from the material shown in Fig. 10B, C indicate that Fe and S are the two major elemental components of these framboidal structures.

Discussion

The vertical distribution of ATP in the water column of the Black Sea showed several consistent and unusual features, including an increase in concentration at the oxic–anoxic interface, concentrations in the deep anoxic zone (>800 m) high relative to those previously reported from oxygenated oceanic environments of comparable depth, and a gradual increase in concentration with depth in the deep anoxic zone. Although these particular features have never been reported from oxygenated seawater, the ATP distribution in the Cariaco Trench (a Caribbean anoxic basin) shows nearly identical vertical trends (Karl et al. 1977). These data suggest that there are factors responsible for controlling the distribution and abundance of microorganisms unique to anoxic basins.

The interface ATP peak is characterized by a very steep gradient (i.e. ΔATP:ΔZ) especially at its upper surface (Figs. 3–6) and by a relatively restricted vertical extent (<20–30 m). From direct observation, Sorokin (1964) reported a similar increase in cells near the oxic–anoxic interface. The ATP peak could result from several different microbial processes. Sorokin also located an active zone of bacterial sulfate reduction directly below the chemocline, and Jannasch et al. (1974) thought this enrichment of heterotrophically growing microorganisms was the result of photosynthetically derived organic material "leaking" through the interface and supplying these obligate

anaerobes with a source of oxidizable organic matter. The large increase in microbial biomass might also be the result of an active sulfide-oxidizing bacterial population. Sorokin (1964, 1970) measured high rates of reduction of $[^{14}C]CO_2$ in the absence of light slightly above the interface region, a process that he interpreted as "chemosynthesis," and observed microscopically cells from within the chemocline described as "thiobacillilike." Using a vertical advection-diffusion model, Brewer and Murray (1973) calculated that CO_2, NH_4^+, and HPO_4^{2-} were all consumed within the chemocline of the Black Sea, at rates suggesting active in situ chemosynthetic bacterial production. Tuttle and Jannasch (1973) have isolated sulfide- and thiosulfate-oxidizing bacteria from the interface region of both the Black Sea and the Cariaco Trench, but were very cautious in interpreting the dark carbon dioxide assimilation data, suggesting additional biological and chemical processes that could account for an apparent uptake of $[^{14}C]CO_2$. Most of the sulfide- and thiosulfate-oxidizing bacteria from the Black Sea were facultatively autotrophic, like the organisms isolated from oxygenated oceanic environments (Tuttle and Jannasch 1972). They do not lower the pH as do the sulfate-producing chemolithotrophic thiobacilli, but oxidize reduced sulfur compounds to polythionates. Kuenen (1975) believed there was no unequivocal evidence for the occurrence of true thiobacilli in the Black Sea. Tuttle et al. (1974) have demonstrated growth stimulation by thiosulfate of several heterotrophic marine isolates, suggesting that mixotrophic growth could confer an advantage over purely heterotrophic growth, especially in the ocean where the organic carbon levels are extremely low.

The ATP biomass data presented here show the maximum concentration in that portion of the water column where O_2 and HS^- coexist in very low, but detectable concentrations (Figs. 3–5), rather than distinctly above or below this chemically mixed transition zone. In both of the deep water profiles, the ATP maximum is at an O_2 concentration of 0.2–0.3 $ml \cdot liter^{-1}$ (Figs. 3, 5). These data suggest that most of the organisms at the ATP peak are living aerobically or microaerophilically rather than anaerobically.

The EC ratio (Atkinson and Walton 1967; Atkinson 1969; Chapman et al. 1971) has been used as a linear measure of the amount of metabolic energy stored in the adenine nucleotide pools (Swedes et al. 1975). Extensive laboratory studies indicate that the EC in growing cells is stabilized at a value of 0.8 to 0.9; as growth ceased in pure cultures of *Escherichia coli*, the EC ratio decreased to between 0.8 and 0.5, and when the ratio dropped below 0.5 the cells were no longer viable (Chapman et al. 1971). In principle, the EC ratio might be useful for estimating the overall metabolic state of natural microbial populations (Wiebe and Bancroft 1975) and the potential for cell growth.

The EC data presented in Figs. 3, 4, and 5 indicate that the large increase in biomass in the interface region actually consists of a vertically restricted zone of rapidly growing microbial cells. Although growth rate cannot be determined from EC measurements alone, the rate of protein synthesis and the capacity for growth are more closely coupled to changes in the EC ratio than to changes in the absolute concentration of ATP (Swedes et al. 1975). It is interesting that the maximum concentration of ATP and the maximum EC ratio were both found in the same sample at all three stations (Figs. 3–5). These data support the Egunoff prediction for the existence of a well defined layer of metabolically active bacteria at the Black Sea chemocline. The question that remains unresolved is whether the large biomass and high metabolic activity actually represent the thiobacillilike bacteria proposed by Egunoff or other physiological groups of microorganisms.

Directly below the ATP interface peak there is a broad zone of 200–300 m in vertical extent where both ATP and A_T concentrations decrease rapidly with

depth (Figs. 3, 5). Within the same depth interval, the EC ratio decreases from the maximum value at the chemocline (Figs. 3, 5). It is quite possible that the distribution of microbial biomass and activity within this portion of the water column results from cells passively sinking out of the chemocline where metabolic activity is maximal.

The concentrations of ATP in the deeper portions of the anoxic zone (800–2,100 m) are from 2 to 10 times greater than those from comparable depths in oxygenated oceanic environments (Hobbie et al. 1972; Holm-Hansen and Booth 1966; Holm-Hansen 1969; Karl et al. 1976). The concentrations of ATP, PNA, POC, and PON all increase with depth below 500 m (Figs. 3, 5, 7, Table 1). Previous in situ optical measurements of the intensity of scattered light (Neuimin and Paramonov 1965) and gravimetric determinations of material suspended in the Black Sea (Spencer et al. 1972) show that the levels of particulate material increase with depth within the deep anoxic zone. The deep-water EC values indicate that the biomass increase shown by ATP concentrations below 800–1,000 m is accompanied by an increase in community metabolic activity (Figs. 4, 6).

Although no comprehensive data are available concerning the half-life of nucleic acids in seawater, the considerable DNase activity reported for a coastal marine ecosystem (Maede and Taga 1973) suggests that they are biologically labile. The substantial levels of PNA in the anoxic portion of the Black Sea water column suggest that a significant portion of the total POC is nonrefractory. For example, the total PNA-C at 2,045 m at station 1355 (PNA × 0.34 = PNA-C) accounts for >15% of the total POC (4.8 $\mu g \cdot liter^{-1}$/29.2 $\mu g \cdot liter^{-1}$ = 16.4%). Although there is a very large change in the PNA:ATP ratio with depth (i.e. 70–90 for euphotic zone; 700–900 for deep water), there is a very close correlation between the ATP and PNA concentrations within the anoxic zone. The correlation coefficient (r) calculated for the lower portion of the water column (342–2,045 m) is 0.99, suggesting a direct interaction between the two parameters measured. This increase in nucleic acid-rich material with depth can be discussed in terms of the ATP, A_T, and EC data presented earlier. Rapidly growing bacterial cells contain between 24 and 42% RNA (dry wt basis), and as the growth rate decreased, the intracellular nucleic acid concentration also decreased (Wade and Morgan 1957; Neihardt and Magasanik 1960; Maaloe and Kjeldgaard 1966). The increase in nucleic acid-rich organic material in Black Sea water might result from an actively growing microbial population, as supported by the ATP and EC data, rather than a mere accumulation of nonliving nucleic acids.

Thus far data have been presented that suggest an increase in biomass (ATP), activity (EC), and growth (PNA:ATP ratio) with depth in the anoxic portion of the water column; however, no consideration has been given to the source of energy required for these sustained heterotrophic processes. The Black Sea contains an extremely large pool of DOC, and several investigators have reported a substantial increase with depth in the anoxic portion of the water column (e.g. Datsko *cited in* Richards 1965; Starikova 1970). Deuser (1971) found that the DOC increased from 2 mg $C \cdot liter^{-1}$ at 150 m to a maximum of 6 mg $C \cdot liter^{-1}$ at the bottom (2,100 m), or about 3–8 times greater than values from oxygenated oceanic environments (Menzel and Ryther 1968; Menzel 1970; Williams 1971). This general excess of organic matter in the anoxic portion of the water column has been interpreted by Jannasch et al. (1974) to represent a limitation in the availability of hydrogen acceptors required for further microbial decomposition. One major difficulty with bulk carbon determinations (i.e. total DOC) is the complete lack of information concerning the specific chemical composition (and subsequent biological usefulness) of this material; investigators have now begun to examine the origin, distribution, and utilization or fate of specific classes of organic compounds. The increase in ATP and poten-

tial for cell growth with depth below 800 m in the Black Sea implies the existence of a vertical gradient in specific organic substrates required for bacterial metabolism. Fermentation reactions within the organic-rich sediments, combined with upward vertical advection and diffusion processes, are the primary factors responsible for maintaining these gradients. The similar situation in the anoxic portion of the Cariaco Trench (Karl et al. 1977) suggests that similar chemical and microbiological features may be typical of all oceanic anoxic basins.

The average concentration of ATP within the uppermost sediment layers (0–10 cm) is about 80 and 150 ng ATP·cm^{-3} wet sediment at stations 1355a (2,200 m) and 1354 (450 m) with a greater subsurface peak at the shallow water station (Fig. 8). These ATP levels are comparable with those in sediments from 80 km off the coast of Spanish Sahara in 1,000 m of water (Hodson et al. 1976) and in shallow sediments (<50 m) about 6–10 km offshore in the Southern California Bight (Karl unpublished data), but are nearly 100 times greater than ATP values in a deep oceanic sample (6,000 m) collected in the Nares Abyssal Plain (Karl et al. 1976). These differences may reflect the availability of organic matter in these ecosystems. Recent Black Sea sediments are extremely rich in organic carbon and may contain >15% organic material by weight (Degens et al. 1970; Hunt 1974; Degens and Ross 1974). Dissolved organic carbon concentrations in the interstitial waters of Black Sea sediment cores are several times greater than the DOC in the overlying water column, ranging from 7.2 to 62.6 mg C·liter^{-1} depending on the amount of particulate organic matter present (Starikova 1970).

One consistent feature of the vertical profiles of ATP was that subsurface peaks were found at a depth of about 3–6 cm in the core, rather than as expected directly at the water–sediment interface. Since the Black Sea is anoxic from about 125–225 m to the bottom and throughout the entire core, the observed distribution is not likely to be caused by strong gradients in dissolved gases, nutrients, pH, or redox potential. One striking physical feature of the Black Sea sediments was the existence of a fluid mud, which has been described as a "fluffy gelatinous-appearing aggregation composed primarily of detrital minerals with small amounts of both particulate organic matter and biogenic carbonate materials" (Barnard and Fanning pers. comm.). The water content of this layer was very high (≈96%), resulting in an extremely low sediment density (1.049 g·cm^{-3}: Barnard and Fanning pers. comm.). Since the values are calculated per volume of wet sediment, subsurface peaks may simply be the result of this large vertical variation in sediment density.

Although direct involvement of bacteria in the formation of sedimentary deposits of native sulfur cannot be disputed (Ivanov 1968; Sokolova and Karavaiko 1968), the question of the importance of microorganisms in the diagenesis of iron sulfide minerals (particularly pyrite) is still open. Several obvious (and relatively undisputed) indirect roles of bacteria in the formation of authigenic iron sulfide are: maintenance of anoxic conditions through removal of O_2 by the metabolic activity of facultative anaerobic bacteria; maintenance of proper pH and redox potential levels to ensure thermodynamic stability of the appropriate mineral phases; and reduction of SO_4^{2-} to HS^- via anaerobic respiration. Reports of pyrite formation in oxidizing sediments (Emery and Rittenburg 1952) and in the tests of living Foraminifera (Seiglie 1973) both suggest microbial involvement. The direct evidence is based primarily on observations that laboratory cultures of sulfate-reducing bacteria can precipitate iron sulfides either within their cell walls (Issatschenko *cited in* ZoBell and Rittenburg 1948) or as an external cell coating (Hallberg 1965). A recent report that microbiologically formed iron sulfide minerals showed no chemical, textural, or crystallographic differences from sulfides precipitated inorganically (Rickard 1969)

suggests that the direct chemical reactions of formation are independent of bacterial activities.

The scanning electron micrographs presented in Fig. 10 depict what I consider to be the early stages in the diagenesis of framboidal iron sulfide. Sweeney and Kaplan (1973) suggested that the spherical texture is developed when the initial iron monosulfide precipitate is transformed into greigite (cubic Fe_3S_4), and as greigite is further transformed into pyrite (FeS_2), internal nucleation occurs to form the familiar framboidal structure. In contrast to previously published scanning electron micrographs of iron sulfide framboids (Sweeney and Kaplan 1973; Goldhaber and Kaplan 1974; Degens and Ross 1974) those shown here reveal either intimate association with recognizable bacterial colonies (Fig. 10A), or biologically derived mucuslike threads (Fig. 10B, C) connecting individual microcrystallites (bacterial cells?). In addition, Fig. 10C shows a nonspherical aggregate displaying some characteristics of a bacterial colony (mucuslike accretions, cell size 0.7 μ, proximity to former biological material) and some characteristics of authigenic pyrite (microcrystallite size 0.5–1.0 μ, framboidal appearance, but not spherical).

The concentration of iron dissolved in the interstitial waters of Black Sea sediments is extremely high (0.4–6 mg·liter^{-1}: Manheim and Chan 1974), so it is not unlikely that iron sulfide would precipitate around localized sources of HS^- (i.e. sulfate-reducing bacteria). Although some earlier work has de-emphasized the importance of bacteria (Vallentyne 1963; Rickard 1969, 1970; Farrand 1970), no comparisons have been made of the kinetics of iron sulfide precipitation with and without the presence of bacteria. The actual role of microorganisms in the authigenic formation of metal sulfides may be related to their ability to serve as catalytic sites for inorganic precipitation. A similar role of living catalyst has been proposed for certain bacteria in the formation of deep-sea manganese nodules (Crerar and Barnes 1974). To evaluate these hypotheses, we need to conduct in situ experiments so that the exact physicochemical reaction conditions in natural systems can be duplicated.

References

ATKINSON, D. E. 1969. Regulation of enzyme function. Annu. Rev. Microbiol. **23:** 47–68.

———, AND G. M. WALTON. 1967. Adenosine triphosphate conservation in metabolic regulation. J. Biol. Chem. **242:** 3239–3241.

BODMAN, R. H., L. W. SLABAUGH, AND V. T. BOWEN. 1961. A multi-purpose large volume seawater sampler. J. Mar. Res. **19:** 141–148.

BREWER, P. G., AND J. W. MURRAY. 1973. Carbon, nitrogen and phosphorus in the Black Sea. Deep-Sea Res. **20:** 803–818.

CARLUCCI, A. F., S. L. SHIMP, P. A. JUMARS, AND H. W. PAERL. 1976. In situ morphologies of deep-sea and sediment bacteria. Can. J. Microbiol. **22:** 1667–1671.

CASTELLVI, J. 1975. Autotrophic and heterotrophic processes in the activity of marine bacteria [in Spanish, English summary]. Invest. Pesq. **39:** 119–144.

CHAPMAN, A. G., L. FALL, AND D. E. ATKINSON. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. **108:** 1072–1086.

CRERAR, D. A., AND H. L. BARNES. 1974. Deposition of deep-sea manganese nodules. Geochim. Cosmochim. Acta **38:** 270–300.

DEGENS, E. T., AND D. A. ROSS [EDS.]. 1974. The Black Sea—geology, chemistry and biology. Am. Assoc. Pet. Geol. Mem. 20. 633 p.

———, S. W. WATSON, AND C. C. REMSEN. 1970. Fossil membranes and cell wall fragments from a 7000-year-old Black Sea Sediment. Science **168:** 1207–1208.

DEUSER, W. G. 1971. Organic carbon budget of the Black Sea. Deep-Sea Res. **18:** 995–1004.

EMERY, K. O., AND S. C. RITTENBURG. 1952. Early diagenesis of California basin sediments in relation to origin of oil. Am. Assoc. Pet. Geol. Bull. **36:** 735–806.

FARRAND, M. 1970. Framboidal sulphides precipitated synthetically. Miner. Deposita **5:** 237–247.

GOLDHABER, M. B., AND I. R. KAPLAN. 1974. The sulfur cycle, p. 569–655. *In* E. D. Goldberg [ed.], The sea, v. 5. Wiley-Interscience.

HALLBERG, R. 1965. Notes on the biosynthesis of pyrite. Stockholm Contrib. Geol. **13:** 35–37.

HOBBIE, J. E., AND OTHERS. 1972. A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. **17:** 554–555.

HODSON, R. E., O. HOLM-HANSEN, AND F. AZAM. 1976. Improved methodology for ATP determinations in marine environments. Mar. Biol. **34:** 143–149.

HOLM-HANSEN, O. 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. **14:** 740–747.

———. 1973. Determination of total microbial biomass by measurement of adenosine triphosphate, p. 73–89. *In* L. H. Stevenson and R. R. Colwell [eds.], Estuarine microbial ecology. Univ. South Carolina Press.

———, AND C. R. BOOTH. 1966. The measurement of ATP in the ocean and its ecological significance. Limnol. Oceanogr. **11:** 510–519.

HUNT, J. M. Hydrocarbon geochemistry of the Black Sea, p. 499–504. *In* E. T. Degens and D. A. Ross [eds.], The Black Sea—geology, chemistry and biology. Am. Assoc. Pet. Geol. Mem. 20.

IVANOV, M. V. 1968. Microbiological processes in the formation of sulfur deposits. Israel Program Sci. Transl. Jerusalem.

JANNASCH, H. W., H. G. TRUPER, AND J. H. TUTTLE. 1974. Microbial sulfur cycle in Black Sea, p. 419–425. *In* E. T. Degens and D. A. Ross [eds.], The Black Sea—geology, chemistry and biology. Am. Assoc. Pet. Geol. Mem. 20.

KARL, D. M., AND O. HOLM-HANSEN. In press. Methodology and measurement of adenylate energy charge ratios in environmental samples. Mar. Biol.

———, AND P. A. LAROCK. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Bd. Can. **32:** 599–607.

———, ———, J. W. MORSE, AND W. STURGES. 1976. Adenosine triphosphate in the North Atlantic Ocean and its relationship to the oxygen minimum. Deep-Sea Res. **23:** 81–88.

———, ———, AND D. J. SHULTZ. 1977. Adenosine triphosphate and organic carbon in the Cariaco Trench. Deep-Sea Res. **24:** 105–113.

KRISS, A. E. 1959. The role of microorganisms in the primary production of the Black Sea. J. Cons., Cons. Int. Explor. Mer **24:** 221–230.

KUENEN, J. G. 1975. Colorless sulfur bacteria and their role in the sulfur cycle. Plant Soil **43:** 49–76.

MAALOE, O., AND N. O. KJELDGAARD. 1966. Control of macromolecular synthesis. Benjamin.

MAEDE, M., AND N. TAGA. 1973. Deoxyribonuclease activity in seawater and sediment. Mar. Biol. **20:** 58–63.

MANHEIM, F. T., AND K. M. CHAN. 1974. Interstitial waters of Black Sea sediments: New data and review, p. 155–180. *In* E. T. Degens and D. A. Ross [eds.], The Black Sea—geology, chemistry and biology. Am. Assoc. Pet. Geol. Mem. 20.

MENZEL, D. W. 1970. The role of in situ decomposition of organic matter on the concentration of non-conservative properties in the sea. Deep-Sea Res. **17:** 751–764.

———, AND J. H. RYTHER. 1968. Organic carbon and the oxygen minimum in the South Atlantic Ocean. Deep-Sea Res. **15:** 327–337.

NEIDHARDT, F. C., AND B. MAGASANIK. 1960. Studies on the role of RNA in the growth of bacteria. Biochim. Biophys. Acta **42:** 99–116.

NEUIMIN, G. G., AND A. N. PARAMONOV. 1965. Distribution of suspended matter in the deep-water regions of the Black Sea. Akad. Nauk SSSR Izv., Atmos. Oceanol. Phys. Ser. **1:** 696–699.

PAERL, H. W., AND S. L. SHIMP. 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnol. Oceanogr. **18:** 802–805.

PRADET, A. 1967. Étude des adénosine-5-mono di et tri-phosphates daus les tissus végétaux. 1. Dosage enzymatique [in French, English summary]. Physiol. Veg. **5:** 209–221.

RAVIC-SCERBO, I. A. 1930. To the question of bacterial thin layer in the Black Sea according to the hypothesis of Prof. Egunoff [in Russian, English summary]. Akad. Nauk SSSR, Tr. Sevast. Biol. Sta. **2:** 127–141.

RICHARDS, F. A. 1965. Anoxic basins and fjords, p. 611–645. *In* J. P. Riley and G. Skirrow [eds.], Chemical oceanography, v. 1. Academic.

RICKARD, D. T. 1969. The microbiological formation of iron sulphides. Stockholm Contrib. Geol. **20:** 49–66.

———. 1970. The origin of framboids. Lithos **3:** 269–293.

SCHNEIDER, W. C. 1957. Determination of nucleic acids in tissues by pentose analysis, p. 680–684. *In* Methods in enzymology, v. 3. Academic.

SEIGLIE, G. A. 1973. Pyritization in living foraminifers. J. Foraminiferal Res. **3:** 1–6.

SEN GUPTA, R., AND H. W. JANNASCH. 1973. Photosynthetic production and dark-assimilation of CO_2 in the Black Sea. Int. Rev. Gesamten Hydrobiol. **58:** 625–632.

SOKOLOVA, G. A., AND G. I. KARAVAIKO. 1968. Physiology and geochemical activity of Thiobacilli. Israel Program Sci. Transl. Jerusalem.

SOROKIN, J. I. 1964. On the primary production and bacterial activities in the Black Sea. J. Cons., Cons. Int. Explor. Mer **29:** 41–60.

———. 1970. Experimental investigation of the rate and mechanism of oxidation of hydrogen sulfide in the Black Sea using S-35. Oceanology **10:** 37–46.

SPENCER, D. W., P. G. BREWER, AND P. L. SACHS. 1972. Aspects of the distribution and trace element composition of suspended matter in the Black Sea. Geochim. Cosmochim. Acta **36:** 71–86.

STARIKOVA, N. D. 1970. Vertical distribution patterns of dissolved organic carbon in sea water and interstitial solutions. Oceanology **10:** 796–807.

SUTCLIFFE, W. H., E. A. ORR, AND O. HOLM-HANSEN. 1976. Difficulties with ATP measurements in inshore waters. Limnol. Oceanogr. **21:** 145–149.

SWEDES, J. S., R. J. SEDO, AND D. E. ATKINSON. 1975. Relation of growth and protein synthesis to the adenylate energy charge in an adenine-

requiring mutant of *Escherichia coli*. J. Biol. Chem. **250:** 6930–6938.

SWEENEY, R. E., AND I. R. KAPLAN. 1973. Pyrite framboid formation: Laboratory synthesis and marine sediments. Econ. Geol. **68:** 618–634.

TUTTLE, J. H., P. E. HOLMES, AND H. W. JANNASCH. 1974. Growth rate stimulation of marine pseudomonads by thiosulfate. Arch. Microbiol. **99:** 1–14.

———, AND H. W. JANNASCH. 1972. Occurrence and types of thiobacillus-like bacteria in the sea. Limnol. Oceanogr. **17:** 532–543.

———, AND ———. 1973. Sulfide and thiosulfate-oxidizing bacteria in anoxic marine basins. Mar. Biol. **20:** 64–70.

VALLENTYNE, J. R. 1963. Isolation of pyrite spherules from recent sediments. Limnol. Oceanogr. **8:** 16–30.

WADE, H. E., AND D. M. MORGAN. 1957. The nature of the fluctuating ribonucleic acid in *Escherichia coli*. Biochem. J. **65:** 321–331.

WIEBE, W. J., AND K. BANCROFT. 1975. Use of adenylate energy charge ratio to measure growth state of natural microbial communities. Proc. Natl. Acad. Sci. **72:** 2112–2115.

WILLIAMS, P. M. 1971. The distribution and cycling of organic matter in the ocean, p. 145–160. *In* S. O. Faust and J. V. Hunter [eds.], Organic compounds in aquatic environments. Dekker.

ZAJIC, J. E. 1969. Microbial biogeochemistry. Academic.

ZOBELL, C. E. 1946. Marine microbiology. Chron. Bot.

———, AND S. C. RITTENBURG. 1948. Sulfate-reducing bacteria in marine sediments. J. Mar. Res. **7:** 602–617.

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