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Microbial biomass and population diversity in the upper water column of the Black Sea

DAVID F. BIRD*† and DAVID M. KARL*‡

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Abstract—Estimates of bacterial abundance, bacterial biomass and microplankton biomass in the Black Sea were made in the upper portion of the water column (0–500 m) during the second leg (14–28 May 1988) of the U.S.–Turkey Expedition aboard R.V. *Knorr*. The microbial biomass maximum at the interface between oxygenated and sulfide-rich waters, seen in previous years in the Black Sea and generally characteristic of redoxclines in other anoxic marine habitats, was absent on this occasion. In spite of the absence of a general enrichment in total microbial or bacterial biomass across the oxic-to-anoxic boundary, there were sharply delineated populations of microorganisms living at specific depths within the redoxcline. Among the microbial groups present were phototrophic bacteria, ciliates bearing attached bacteria and putative *Achromatium* cells.

INTRODUCTION

THE presence of oxic–anoxic gradients, or redoxclines, in selected marine environments provides a potential habitat for the growth and accumulation of specialized microbial communities (JØRGENSEN, 1982). Redoxclines in sea water range in dimension from microscopic (PAERL, 1985), through the narrow (<3 mm) but intensely active interfaces of microbial mats (JØRGENSEN, 1982), to regions of extensive (>10 m) co-occurrence of both O_2 and H_2S (SOROKIN, 1964; KARL, 1978). The region of the water column where both O_2 and H_2S co-exist has been termed the "C-layer" (co-existence layer; FASHCHUK and AYZATULLIN, 1986).

Investigations of the distribution and abundance of microorganisms within the vicinity of stable O_2–H_2S redoxclines has generally revealed an enrichment of cell numbers and cell biomass at or slightly above the region where H_2S is first detected ($H_2S = 0.5$–$1\,\mu M$). If the O_2–H_2S boundary is within the euphotic zone, the microbial community may be dominated by phototrophic sulfur bacteria (SOROKIN, 1965; INDREBØ *et al.*, 1979; SORENSEN, 1988); otherwise chemoautotrophs, including H_2S, NH_4^+ and CH_4 oxidizing bacteria, are thought to comprise a substantial portion of the microbial assemblage (SOROKIN, 1962, 1972, 1983).

* School of Ocean and Earth Science and Technology, Department of Oceanography, University of Hawaii, Honolulu, HI 96822, U.S.A.

† Present address: Department of Biological Sciences, UQAM, P.O. Box 8888, Succursale A, Montreal, Quebec, Canada 43C 3P8.

‡ Author to whom correspondence should be addressed.

These natural enrichments of chemoautotropic bacteria are ultimately dependent upon a continued supply of reduced substrates from the deeper anoxic portion of the water column and an appropriate electron acceptor (e.g. O_2 or NO_3) from the oxygenated surface waters. In permanently stratified marine habitats (e.g. Black Sea, Cariaco Trench, Framvaren Fjord), enrichments of microorganisms at oxic–anoxic interfaces have been quantified by both traditional tools of light microscopy and enrichment culture (KRISS, 1959, 1963; SOROKIN, 1962; MITSKEVICH, 1979; ORMEROD, 1988) and by measurements of adenosine triphosphate (ATP) as an independent indicator of living microbial biomass accumulation (KARL *et al.*, 1977; KARL, 1978; ORMEROD, 1988). Microbial population enrichments (expressed as peak concentrations within the redoxcline relative to mean values well above or below the discontinuity) were, however, quite variable, ranging from factors of less than 2-fold (KARL, 1978) for ATP concentrations in the Black Sea to greater than 10-fold in the Framvaren Fjord (SORENSEN, 1988). To a certain extent, these comparisons may be somewhat subjective owing to the characteristically stratified nature of the bacterial enrichments in relation to the chemical gradients in a particular habitat. Sampling and, in particular, sample spacing most likely will define the vertical population structure and the apparent magnitude of the measured enrichments (if any) thereof. Nevertheless, there is ample evidence to suggest that microbial and bacterial biomass is elevated at or near redox level discontinuities.

During the past decade, dramatic changes have taken place in the characteristics of the Black Sea chemocline (MURRAY *et al.*, 1989). First, the anoxic, sulfide-containing zone has shoaled by perhaps as much as 30 m (FASHCHUK and AYZATULLIN, 1986; LEONOV and AYZATULLIN, 1987; MURRAY *et al.*, 1989). Even more intriguing however, was the presence of a significant gap of 10–40 m between O_2-containing (>5 μM) and H_2S-containing (>1 μM) waters (MURRAY *et al.*, 1989). The C-layer was notably absent. If the enrichment of chemoautotrophic bacteria is dependent upon adequate concentrations of both electron donors and electron acceptors, then one might predict that microbial biomass in the suboxic zone would be low, or at least not enriched relative to other portions of the redoxcline.

The objective of this study was, first of all, to provide direct estimates of the standing stock of microorganisms in the upper water column of the Black Sea (0–500 m), from the surface through the suboxic region and into the upper sulfide-rich layers of the permanently anoxic zone. For this portion of the study we employed three independent and, therefore, redundant estimates of microbial biomass: direct microscopy, ATP and lipopolysaccharide (LPS) concentrations. These estimates also provide complementary information to concurrent measurements of vertical flux and microbial production (KARL and KNAUER, 1991) and microbial sulfur oxidation (JØRGENSEN *et al.*, 1991). Second, we were interested in enumerating the protistan populations near the redoxcline either to identify anaerobic protists exploiting the potential bacterial production in the oxic–anoxic interface, or if protozoans were absent, to examine a bacterioplankton population free from the influence of phagotrophic grazing pressures.

METHODS

Samples were collected on the second cruise of the joint U.S.–Turkey Oceanographic Expedition aboard R.V. *Knorr*, on 14–28 May 1988 (*Knorr* cruise 134, leg 9). The emphasis during this cruise was on the microbiology of the carbon, sulfur and nitrogen

cycles, with special attention paid to fluxes occurring at the oxic–anoxic interface. The three stations sampled were designated BS-21 (42°10′N, 30°00′E), BS-22 (42°50′N, 32°00′E) and BS-23 (43°05′N, 34°00′E).

Water samples for microscopy were collected from 12-place, 30-l Niskin bottle CTD-rosette casts. Twenty-milliliter samples were immediately preserved with 250-μl formalin (1.25% final concentration), and returned to Hawaii for enumeration. Five- to 15-ml subsamples were stained with DAPI (0.5 μg ml^{-1} final concentration; PORTER and FEIG, 1980) and filtered onto 0.2-μm pore-size Nuclepore filters. Bacteria were counted in 30 fields on each filter, so that at least 600 cells were counted per sample. Sulfide zone samples below 125 m were additionally stained with bromophenol blue (10 μg ml^{-1} final concentration) or acridine orange (10 μg ml^{-1} final concentration; HOBBIE *et al.*, 1977) to look for eukaryotes. When stimulated with green light, organisms stained with bromophenol blue fluoresce red, and switching filter sets to the DAPI wavelengths allows examination of nuclear morphology as an aid to identification. For water samples collected in the 0–125 m depth range, eukaryotes were also counted.

The average size of the bacteria present at each depth was determined by measuring the dimensions of 40–50 cells with an ocular micrometer scale. Measurements were made on DAPI-stained cells. In the process of examining the bromophenol blue-stained preparations, it became evident that the DAPI-stained nucleoid of bacteria represents only a fraction of total cell volume. We, therefore, made simultaneous measurements of a range of cells at both DAPI and bromophenol blue wavelengths, and corrected the earlier measurements individually using a regression relationship between DAPI size and "true" size. The equation used was: log cell volume (BB) = 0.34 + 0.85 log cell volume (DAPI); $r^2 = 0.83$, RMS = 0.047 ($n = 48$). The accuracy of these adjusted mean volumes was confirmed at three depths against estimates from acridine orange-stained samples. The underestimate of cell size that results from the use of DAPI has been independently confirmed by other investigators (D. F. Bird, manuscript in preparation). Bacterial carbon was estimated using the conversion factor of 106 fg C μm^{-3} (NAGATA, 1986).

Identification, counting and sizing of eukaryotes were done by size classes. Cells less than 10 μm in diameter were counted in transects at 1000 or 1600×. Cells between 10 and 25 μm in maximum dimension were counted in transects at 400×, covering approximately 30% of the total area of the 25-mm filter. All cells greater than 25 μm on the entire filter were counted, at a magnitude of 400×. Phytoplankton carbon was estimated using the equations of STRATHMANN (1967). Cyanobacteria were counted using the characteristic orange fluorescence of phycoerythrin excited by blue light. Phototrophic bacteria containing photosynthetic pigments were counted using green light excitation in at least 30 fields.

For ATP determinations, water samples (150–1000 ml, depending on depth) were filtered through 25-mm diameter microfine glass fiber filters (Whatman, GF/F), the concentrated particulate materials were extracted in 5 ml of boiling phosphate buffer (pH 7.4, 60 mM; KARL and CRAVEN, 1980) and the extracts were stored frozen (−20°C) for subsequent analysis by the peak height firefly bioluminescence method (KARL and HOLM-HANSEN, 1978).

Subsamples for the measurement of total lipopolysaccharide (LPS) were drawn directly from the 30-l Niskin bottles into clean, pyrogen-free, polyethylene test tubes. The samples were immediately frozen for subsequent determination of LPS by the *Limulus* amoebocyte lysate (LAL; Associates of Cape Cod, Inc.) endotoxin assay (WATSON *et al.*, 1977), using an automated detection system (LAL-5000; Associates of Cape Cod, Inc.).

Commercially-available LPS (Associates of Cape Cod, Inc.) was used as the primary standard.

RESULTS

Oxic–anoxic interface

The overlap between H_2S and O_2-containing waters seen on previous cruises to the Black Sea (e.g. Karl, 1978) was not present during the 1988 R.V. *Knorr* cruise (Murray *et al.*, 1989). The oxic–anoxic boundary had instead apparently moved upward in the water column, and separated into distinct oxygenated, suboxic ($O_2 < 5\ \mu M$) and sulfidic ($H_2S > 1\ \mu M$) zones (Fig. 1).

Microbial biomass

Depth profiles of direct counts of bacterial abundance from the surface oxygenated zone (0–50 m) through the suboxic zone to the upper sulfidic zone (>82 m) are shown in Fig. 1. Profiles were somewhat variable between stations, and between casts within a station. Differences in bacterial abundances between casts were reflected in differences in ATP concentration, however, indicating that variation was probably genuine and not due to

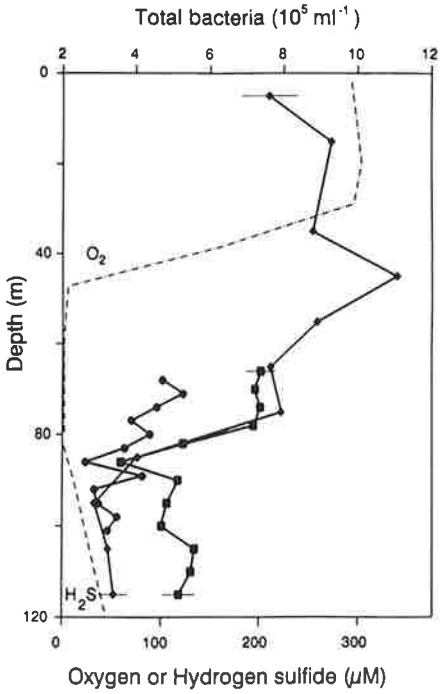


Fig. 1. Bacterial cell abundance in the upper water column at Sta. BS-22, relative to oxygen and hydrogen sulfide concentrations. Chemical data are courtesy of B. B. Jørgensen. Within-sample 95% confidence limits are indicated at top and bottom of profiles only: (●) 17 May profile, (◆) 21 May profile, (■) 26 May profile.

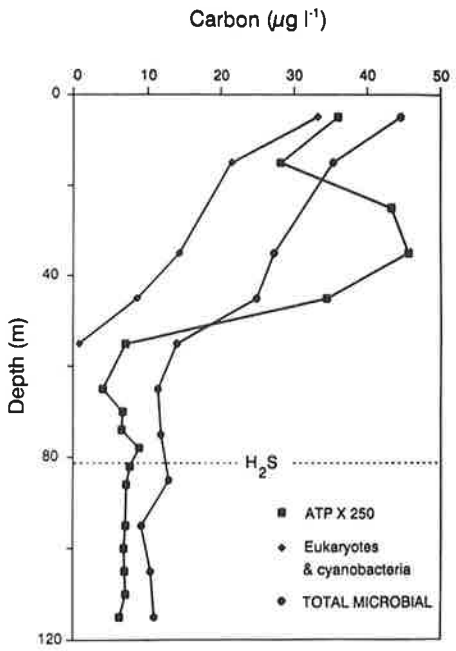


Fig. 2. Microbial biomass profiles from Sta. BS-22. Living biomass has been estimated as particulate ATP concentration × 250 (■). Eukaryote, cyanobacteria and bacteria biomasses were estimated by direct microscopy. Total biomass curve (●) represents eukaryote plus bacterial biomass. ATP curve above 65 m, eukaryote carbon curve, and bacterial curve are from 21 May profile; ATP curve below 65 m is from 26 May profile.

counting artifacts. The mean euphotic zone bacterial abundance (8.5×10^5 cell ml^{-1}) was typical of marine systems of low to moderate productivity (Bird and Kalff, 1984). Euphotic zone abundances were maintained through the suboxic zone on most casts, then fell about 2-fold at the surface of the sulfidic zone. The bacterial biomass peak present at the interface on previous occasions (reviewed in Sorokin, 1983), was notably absent (Fig. 1).

Biomass of the eukaryotic plankton was greatest at the surface (Fig. 2) then declined rapidly with depth. Eukaryotes were virtually absent below 55 m. The phytoplankton assemblage was dominated by dinoflagellates and other flagellates during leg 2. This is the normal community succession following the spring diatom bloom (Bologa *et al.*, 1984). There was a local peak of cyanobacteria (5.2×10^4 per ml) at 35 m. Microbial carbon in the euphotic zone was 1.9 g C m^{-2} estimated from ATP concentration and 1.8 g C m^{-2} from direct microscopic counts and sizing, although the profiles were slightly different in shape (Fig. 2). ATP concentration dropped off rapidly at the bottom of the oxic zone (50 m) whereas bacterial abundance did not. Suboxic and anoxic zone biomass estimated by direct microscopy was greater by a factor of 1.3–1.8 than ATP stocks would predict, suggesting either that a larger portion of the microbiota of deep water were moribund or dead than in surface waters, or that the relationship between cellular ATP and cellular C varied in the two different zones. Nevertheless, the overall agreement between these two independent estimates was remarkable.

There was a radical change in bacterial cell morphology for assemblages restricted to the anoxic zone (depths ≥83 m; Jørgensen *et al.*, 1991). Most of the bacteria in the near-surface waters had a conformation typical of aerobic heterotrophs in marine environments elsewhere; i.e. small in size (mean volume was 0.14 μm^3 $cell^{-1}$) and comparatively uniform in morphology. By contrast, bacteria in the sulfide-rich portion of the water column were diverse in both size and shape. Mean cell volume in the sulfide zone (0.36 μm^3) was more than twice that of cells in the surface waters. This is reflected in the comparison of bacterial abundance (Fig. 1) to bacterial biomass (Fig. 3).

Another independent estimate of bacterial biomass was obtained by measuring the concentration of lipopolysaccharide (LPS), a cell-wall constituent that is unique to Gram negative bacteria, including cyanobacteria and prokaryotic phototrophs (McCartney and Wardlaw, 1985; Weckesser *et al.*, 1979). The profile of total LPS again indicated the absence of any notable peak in bacterial biomass in the region of the redoxcline (Fig. 3). The differences observed in the estimates of bacterial C by LPS vs direct microscopy may be the result of the presence of Gram positive cells (which do not contain LPS) or may be the result of inaccuracies in the conversion factors used to extrapolate biovolume or LPS concentration to cell carbon. Regardless of the cause, the important result is the constancy of both bacterial biovolume and LPS throughout the suboxic and upper sulfidic zones.

Morphological diversity of microorganisms

Direct microscopic examination of the microbial communities revealed the existence of subpopulations of cells associated with specific depth regions, and therefore, presumably, with suitable redox horizons. Most notably, there was a large number of photosynthetic

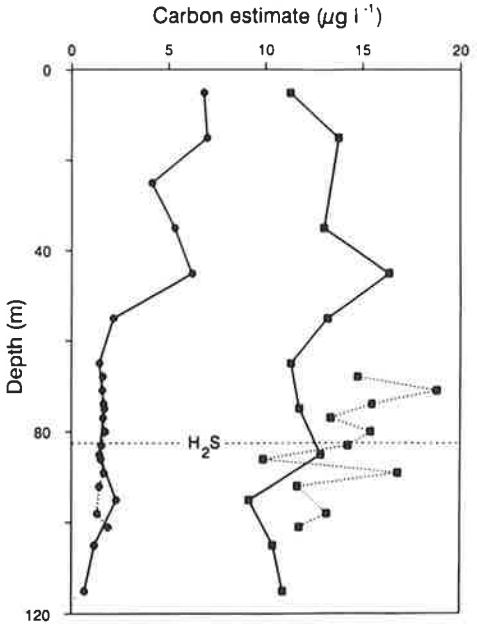


Fig. 3. Profiles of bacterial carbon estimated from LPS concentration (●) and from direct microscopy (■) at Sta. BS-22 for two separate hydrocasts.

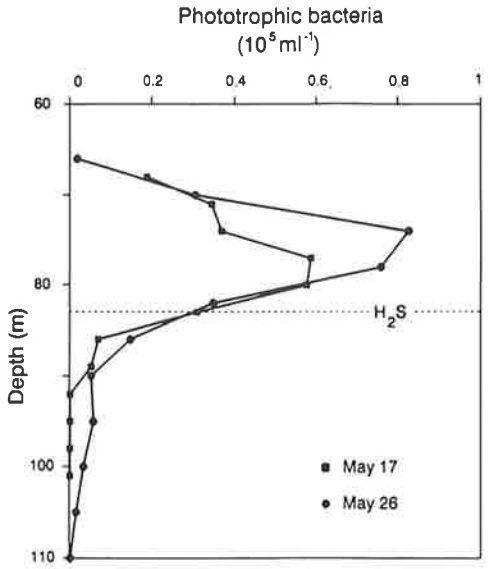


Fig. 4. Profiles of phototrophic bacterial abundance at Sta. BS-22 on two separate hydrocasts: (■) 17 May profile, (●) 26 May profile.

(pigmented) bacteria found between 65 and 90 m in the suboxic zone, at Sta. BS-22 (Fig. 4). These bacteria autofluoresced weakly when excited with green light. Peak abundances occurred at 74–80 m, which we estimate to have been at or below the 0.1% light level (KARL and KNAUER, 1991). Pigmented bacteria at their population density maximum comprised approximately 10–20% of the total bacterial community. The majority of pigmented cells were short (1–3 μm), narrow rods, with rarer half-circle and coccoidal cells. On the basis of high-pressure liquid chromatographic pigment analyses conducted on separate samples collected during leg 2 of the Black Sea cruise it is believed that the community is primarily made up of the green sulfur bacterium, *Chlorobium phaeovibrioides* (SIMPSON and REPETA, 1988; REPETA *et al.*, 1989). Our data provide direct support for the existence and stratification of these phototrophic microorganisms within the suboxic portion of the water column. Pigmented cell abundance declined rapidly at the interface with the sulfidic layer beginning at about 83 m (Fig. 4); few autofluorescent cells could be seen within the sulfide zone.

From 5 to 10% of the microbial biomass within the sulfide zone was made up of rare but very large prokaryotic cells (Fig. 5). The cells were rod-shaped, and appeared to have grown by elongation followed by binary fission. They measured 4–6 μm by 5–11 μm, and were most abundant in the upper sulfidic zone (100–250 m). By morphology alone, these cells appeared to be related to *Achromatium* sp., possibly *A. volutans* (LA RIVIERE and SCHMIDT, 1981), a colorless sulfur-oxidizing bacterium. However, if this population of large cells is actively oxidizing sulfide in the Black Sea, it must be using an electron acceptor other than oxygen.

There was also a sharply delineated layer of ciliates and flagellates living between 76 and 96 m (Fig. 6). The community maximum at 83 m coincided with the top of the sulfide zone. The two ciliate species that made up greater than 90% of the population both carried large

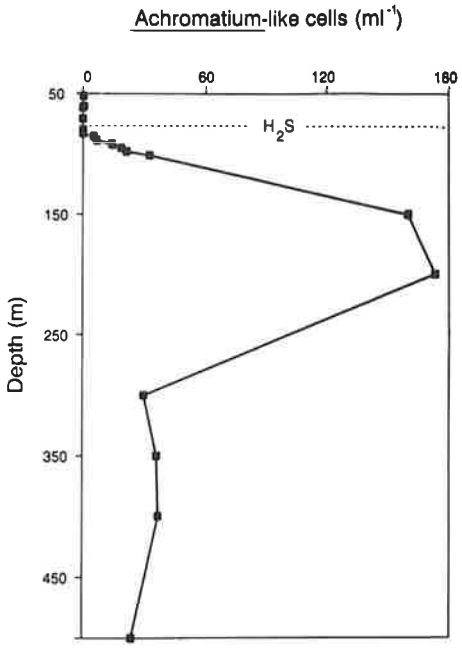


Fig. 5. Composite profile of abundance of *Achromatium*-like cells at Stas BS-22 (17 May) and BS-23 (24 May). No *Achromatium*-like cells were observed at depths shallower than 50 m.

numbers of attached bacteria. The ciliates were small (8–20 μm × 12–23 μm), almond-shaped, and probably belonged to the order Hymenostomatida based on size, shape and ciliation. They could be differentiated by the position and form of the nuclei, one bearing a single central nucleus, the other having an apical nucleus (5 μm) and one micronucleus (2 μm). The size and disposition of bacteria attached to the pellicle also differed. Both organisms carried approximately 50–100 bacteria (0.4–0.6 × 2–4 μm) per cell. The ciliate with the apical micronucleus was usually filled with amorphous brown material that was also evident in the water and presumably had been ingested. Similar inclusions have been seen previously in a ciliate from the sulfidic interface of the Black Sea (Moiseev and Mamaeva, 1980) that was identified as *Pleuronema marinum*.

DISCUSSION

Based on results obtained prior to the 1988 *Knorr* cruise, a fairly consistent picture had emerged regarding the distribution and abundance of microorganisms in the transition zone between oxygenated surface and anoxic deep waters of marine and fresh-water habitats. In marine and fresh-water environments as diverse as Jellyfish Lake, Palau (Burnett *et al.*, 1989), Framvaren Fjord, Norway (Indrebø *et al.*, 1979), meromictic Lake Faro, Sicily (Sorokin and Donato, 1975), the Cariaco Trench, Venezuela (Karl *et al.*, 1977), Big Soda Lake, Nevada (Zehr *et al.*, 1987), Lake Oglethorpe, Georgia (McDonough *et al.*, 1986), Mirror and Knaack Lakes, Wisconsin (Parkin and Brock, 1980) and, historically, the Black Sea itself (reviewed by Sorokin, 1983), microbial biomass has

been shown to exhibit elevated concentrations at or near the redoxcline. The local biomass maximum in these and similar transition layers (e.g. JØRGENSEN, 1982) is usually associated with a production maximum as well.

During leg 2 of the 1988 Black Sea expedition, no local maximum or even generally elevated microbial (based on total ATP) or bacterial (based on total LPS content and direct microscopy) biomass could be observed in the region of the redoxcline (~83 m). Given the historical database, our present results are, therefore, unexpected. While no single method of biomass estimation can be used in ecological studies without a certain level of uncertainty (KARL, 1986), the present use of complementary and redundant analytical procedures gives us an added level of confidence that the profile of microbial biomass is relatively constant through the suboxic zone and into the upper portion of the anoxic zone. Furthermore, given our close sample spacing (approximately 3 m), it is unlikely that a biomass peak escaped our detection. However, in spite of the fact that the chemocline was relatively featureless with respect to microbial biomass, we did observe the expected depth stratification in specific microbial groups (e.g. phototrophic bacteria, *Achromatium*-like bacteria, ciliates). The latter groups did not contribute significantly to total biomass.

TOLMAZIN (1985) and MURRAY *et al.* (1989) hypothesized that dramatic anthropogenic changes are occurring in the hydrology of the Black Sea due to changes in water management of the Dnepr and Dnestr Rivers. The postulated recent rapid rise in the level of the oxic–anoxic interface is supported by Soviet research (FASHCHUK and AYZATULLIN, 1986; NOVOSELOV *et al.*, 1987). Others, however, are not convinced that real change has occurred, but attribute the observed hydrological anomalies to natural variability

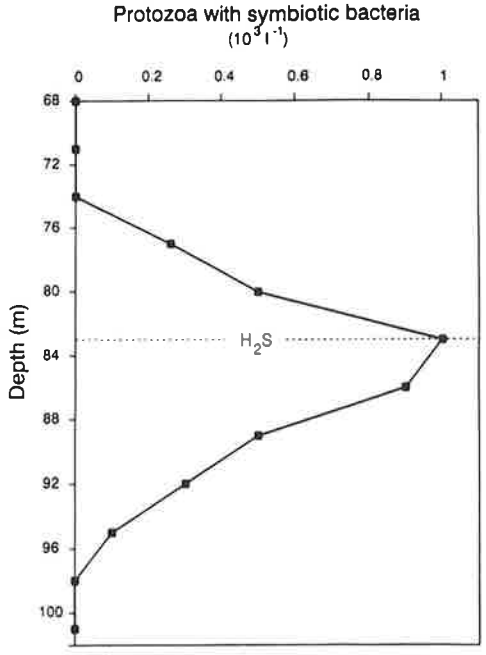


Fig. 6. Abundance of ciliates with attached bacteria found at the suboxic-sulfide layer interface at Sta. BS-22, 17 May profile.

(KEMPE *et al.*, 1990). Recent intensive study of the formation of the cold intermediate layer in the Black Sea showed that this layer forms during convective mixing in the center of cyclonic eddies during winter and early spring (ISAYEVA *et al.*, 1988). The level of the cold intermediate layer can vary by greater than 100 m among cyclonic and anticyclonic eddies. In light of this, the absence of a total biomass peak might be due to mixing. If this were the case, however, we would still need to explain the stratification of specific subpopulations. Furthermore, a profile of bacterial abundance taken during leg 5 of the Black Sea expedition aboard R.V. *Knorr* (July 1988; W. REEBURGH, personal communication) was nearly identical to the one we observed in May, with no evidence for a redoxcline biomass maximum. Perhaps the appearance of a suboxic zone tens of meters in width between oxic and anoxic waters has eliminated the more intense redox discontinuities that must exist when these zones meet or overlap. As a result, populations of diverse metabolic types that formerly co-existed are now physically separated, reduced in size or completely eliminated.

Other aspects of the microbial stratification profile showed striking parallels to previously identified characteristics of anoxic transition zones. The narrow peak of eukaryotes and attendant flora adapted for life in microaerophilic or anoxic conditions can be considered a planktonic sulfide system by analogy to the more elaborate interstitial sulfide system of anoxic marine sediments (FENCHELL and RIEDL, 1970). These habitats contain both obligately and facultatively heterotrophic dinoflagellates, and ciliates with ectosymbiotic bacteria (cf. FENCHEL *et al.*, 1977). The presence in the Black Sea of phototrophic anaerobes and, unexpectedly, of large, "*Achromatium*-like" bacteria are features also common to reducing sediments.

The marked increase in average bacterial cell volume in the sulfide zone is a feature also common to other anoxic habitats. Large increases in cell size associated with anoxia have been seen in the Baltic Sea (GAST and GOCKE, 1988), Big Soda Lake (ZEHR *et al.*, 1987), a small lake in Quebec (BIRD and KALFF, 1989), and the sediments of the Kiel Bight (MEYER-REIL, 1983). This is suggested to result from exclusion of eukaryotic predators from anaerobic regions (MEYER-REIL, 1983). Whereas there were 4700 heterotrophic nanoflagellates per milliliter at 5 m at Sta. BS-22, there were less than 50 per milliliter in the suboxic zone at 65 m, and effectively none at depths below 80 m. Conceivable agents of loss for sulfide zone bacteria include starvation, sedimentation, viral infection and predation by other bacteria.

The existence of photoautotrophic bacteria in the water column of the Black Sea has been a controversial topic. The initial report of KRISS and RUKINA (1953) that they had produced enrichments of phototrophic sulfur bacteria from deep aphotic, anoxic waters has never been substantiated (JANNASCH *et al.*, 1974; HASHWA and TRUPER, 1978), and has therefore been considered mistaken (HASHWA and TRUPER, 1978). HASHWA and TRUPER (1978) did isolate *Chromatium warmingii* and *Thiocapsa roseopersicina* from aphotic bottom sediments, where the cells were probably subsisting fermentatively with no known possibility of reproduction. DICKMAN and ARTUZ (1978) found large accumulations of *Thiocapsa roseopersicina* at 160 and 200 m in the fall of 1977, containing 3–4 times as much biomass as the *Chlorobium* peak found on the present expedition. Our estimates of contemporary light availability in the Black Sea indicate that so little light penetrates to these deep layers that photosynthesis must be minimal, even by *Chlorobium* species, which are among the most efficient photoautotrophs at low light levels (BIEBL and PFENNIG, 1978; VAN GEMERDEN, 1983). Standard figures for light availability at the latitude

of the Black Sea (about 16 ly h^{-1} at photosynthetically active wavelengths; GATES, 1980) in conjunction with our Secchi disk measurement of 22 m (cf. PARSONS *et al.*, 1984, p. 69) indicate that light at the pigmented cell peak (78 m) was probably at most 0.04 ly h^{-1}, or roughly 0.2% of surface value. No light-stimulated reduction of CO_2 or oxidation of H_2S was found in this region (KARL and KNAUER, 1991; JØRGENSEN *et al.*, 1991) even though, in theory *Chlorobium phaeovibrioides* could grow, albeit slowly (generation time >14 days), in this light regime (BIEBL and PFENNIG, 1978). Neither KARL and KNAUER (1991) nor JØRGENSEN *et al.* (1991) would have been able to detect these low rates of growth given their respective experimental designs. Their results, however, are consistent with the low light and low H_2S fluxes in this suboxic habitat.

The abundance of *Chlorobium* we found was sufficient to account for most or all of the bacteriochlorophyll *e* observed by SIMPSON and REPETA (1988) above the sulfide zone, given the capacity of the Chlorobiaceae to increase specific light-harvesting pigment content at low light levels. The pigment profile of SIMPSON and REPETA (1988) showed large amounts of Bchl *e* below the 83-m interface as well, where we did not see more than trivial numbers of cells. It may have been that the bacteriochlorophyll of this zone was detrital and was being preserved due to the absence of oxygen.

In summary, the unusual hydrographic profile found on the joint U.S.–Turkey expedition to the Black Sea, that moved MURRAY *et al.* (1989) to suggest that major anthropogenic influences were at work, seems to be consistent with an equally unusual distribution and abundance of bacteria. The characteristic peak in microbial biomass which is commonly observed at aquatic redoxclines was not present on this occasion. Distinct subpopulations associated with specific depths were seen, however. There was a narrow band of phototrophic bacteria just above the sulfide layer. It accounted for 20% of the bacterial community at its peak. Together with flagellates, ciliates and normally benthic species of bacteria, they formed a qualitatively distinct biome similar to the one recognized in anoxic sediments, albeit a somewhat taxonomically impoverished one.

Away from the interface, the anaerobic bacterial community was notable for its size and evident diversity. The full extent and significance of this diversity could not, of course, be addressed with the microscope alone, and remains a major unknown in the microbial ecology of the Black Sea. The minimal metabolic activity of the sulfide zone bacteria shown by their negligible effect on sinking organic matter (KARL and KNAUER, 1991), in conjunction with the paucity of eukaryotic predators found in this study, suggests a physiologically arrested community. The slowly or sporadically growing cells are allowed to accumulate to biomass levels several times greater than the euphotic zone community they depend on, because removal rates are extremely low.

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