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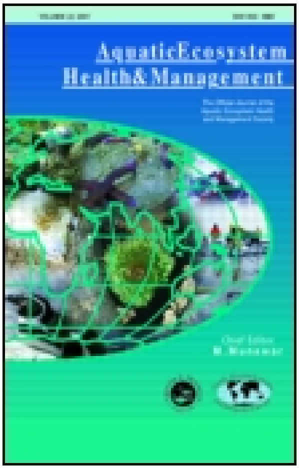

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The dynamics of nutrient enrichment and primary production related to recent changes in the ecosystem of the Black Sea

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Abstract

The data collected in the southern Black Sea during the period of July 1997 to September 1998 showed that light penetrated into the upper 20-35 m, with a downward attenuation coefficient varying between 0.1 and 0.25 m^{-1}. The chlorophyll-a concentration for the euphotic zone ranged from <0.5 to 1.5 $\mu g\ l^{-1}$. Coherent sub-surface chlorophyll-a maxima formed near the base of the euphotic zone at all study points and a secondary one was observed at very low level of light (<0.1% of the surface light) in the nearshore regions. Production rates were estimated in between 62 and 785 $mg\ C\ m^{-2}\ d^{-1}$ in this period. Bioassay experiments (using extra nitrate, ammonia, phosphate, silicon and iron) showed that under optimum light conditions the phytoplankton population is nitrate limited in open waters. Phosphate seems to control the growth in the nearshore regions of the southern Black Sea. Silicon concentration also influenced the phytoplankton growth since the majority of the population was determined to be diatoms.

Keywords: nutrient availability, phytoplankton activity, ecosystem changes, Black Sea

1. Introduction

The Black Sea is a relatively large, deep, landlocked basin with deep-water anoxia and there exists a permanent and strong halocline at depths of >50-200 m, shoaling in the central cyclones and deepening in the coastal regions. Continuous downward transport of bio-genic particles from the productive surface layer, combined with limited vertical ventilation through the permanent halocline are the major reasons for the anoxic conditions. The presence of a cyclonically meandering rim current along the peripheries of the basin partly isolates coastal waters from the interior waters. The Black Sea is further unique in possessing a very narrow shelf along more than half of its margin, except in the north-western area. In addition to natural biochemical processes driving the anoxia in the deep waters, the increasing input of nutrients and organic matter from land-based sources during the last two decades, have generated dramatic changes in the Black Sea ecosystem, especially in the wide north western shelf (Bologa, 1985/1986; Mee, 1992; Bologa et al., 1995; Cociasu et al., 1996, 1997). Long-term modifications and collapses of the biological structure of the ecosystem have been well documented (Shuskina and Musaeva, 1990; Bologa et al., 1995). Additionally, comparison of the earlier measurements with the high-resolution data obtained since 1988 has enabled several workers to address the magnitude of the long-term changes in the nutrient and oxygen profiles from the upper layer down to the sulphide-bearing waters (Murray et al., 1989, 1991, 1995; Codispoti et al., 1991; Tugrul et al., 1992; Basturk et al., 1994). The changes were more significant for the north-western shelf in terms of nutrient input (both in quantity and quality) and the nutrient chemistry of the waters itself (Cociasu et al., 1997) and in terms of biological life (Bologa et al., 1995).

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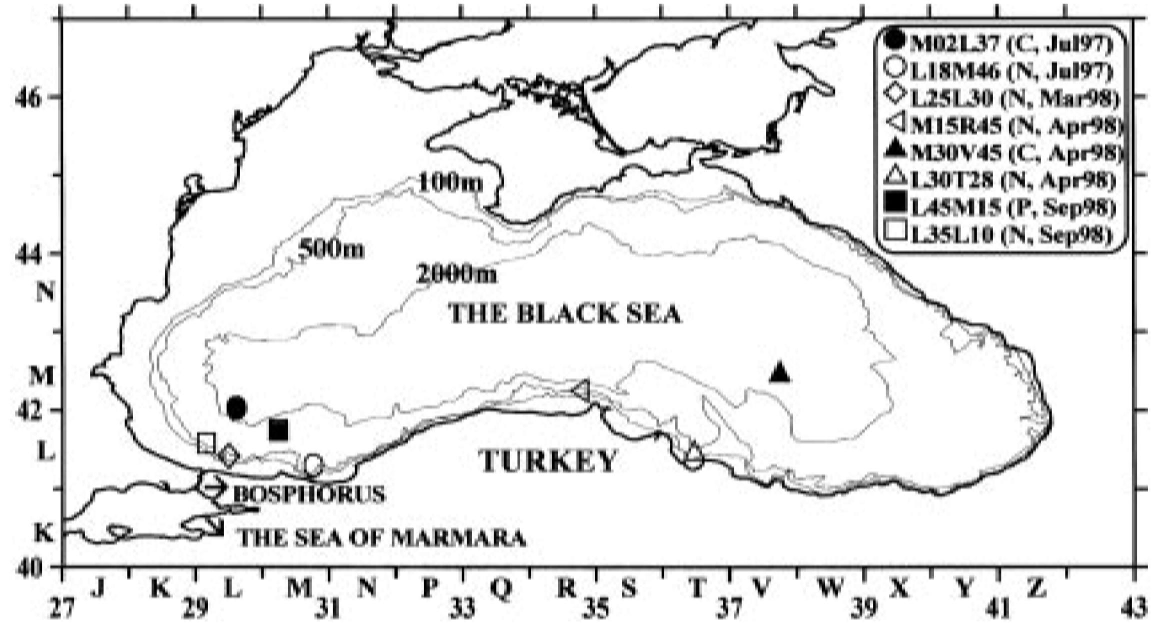


Figure 1 Station network for the cruises in the southern Black Sea during July, 1997-September, 1988 period. Open symbols denote the stations in the nearshore (N) region including the rim current while the filled ones represent the stations located in the central cyclones (C). One of the stations (the deep one, which is symbolised by filled square) is considered to be located in the peripheral (P) area between the rim current and western central cyclone for September 1998 cruise.

The upper cold intermediate layer (CIL) down to the temperature minimum depth in the rim current region is enriched with nitrate but drastically poor in phosphate. There are thus very high nitrate to phosphate ratios in the upper nutricline and an apparent shift in the nutricline onset in the CIL (Basturk et al., 1997). Since these P-limited CIL waters are mixed vertically with the surface waters in winter and early spring, blooms in such areas are mostly limited by P. One should also note that the NO_3/PO_4 ratio has significantly increased in the riverine input in the last decades; for example it was reported as 11.7 before 1970s (Almazo, 1961, cited in Cociasu et al., 1996) and as 22-33 for 1988-1992 period (Cociasu et al., 1996) in the Danube waters from which 70% of the total discharge in the Black Sea is provided.

Nitrate-limited production occurs in the central gyres due the low NO_3 to PO_4 ratios of the chemocline established just below the euphotic zone (Basturk et al., 1997). The relatively high atomic N/P ratios of particulate organic matter (POM) indicate that the anomalously low ratio of NO_3/PO_4 in the oxic/anoxic interface of the entire deep basin is due to NO_3 removal via denitrification, greatly exceeding P-export from the suboxic waters (Yilmaz et al., 1998b).

Changes recently observed in the Black Sea such as the reduction in dissolved silicate load of the Danube River by about two-thirds since dam constructions in the early 1970s, concomitant decrease in wintertime Si concentration by more than 60% in the central areas (Humborg et al., 1997), increase in NO_3concentrations in the north-western shelf waters (Cociasu et al., 1996) and above the pycnocline in the whole Black Sea (Codispoti et al., 1991; Tugrul et al., 1992) significantly influenced the ecosystem of the lower trophic levels.

Primary production in the Black Sea results in two phytoplankton maxima throughout the year; the major one occurs in early spring while a secondary peak appears in autumn (Vedernikov and Demidov, 1993). Recently, additional summer blooms have frequently been observed in both the coastal and open waters (Hay et al., 1990; Yilmaz et al., 1998a, b). Primary production is relatively low in the open sea (50-200 g C m^{-2} y^{-1}) (Vedernikov and Demidov, 1993) compared to the northwestern shelf area (up to 400 g C m^{-2} y^{-1}) (Bologa, 1985/1986; Bologa et al., 1995). Input of nutrients from the anoxic layer (AL) through the permanent pycnocline is limited both by denitrification and by oxidation-reduction processes occurring in the oxic/anoxic transition layer, since the major nutrient source for the open system is the input from the nutricline (Yilmaz et al., 1998b). New production in the open waters of the Black Sea is therefore dominated by the input from the nutricline, riverine input via surface circulations. Since atmospheric transport is probably of secondary importance, new production in the Black Sea is low (Murray et al., 1995; Oguz et al., 1996).

The main purpose of the present study is to understand the key biochemical processes in the upper water column related to primary production in the Black Sea. Interactions between the physical, optical, chemical and biological processes of the upper water column involving nutrient transportation, the uptake mechanisms of nutrients by primary producers and the determination of the growth capacity of phytoplankton populations living in the Black Sea were investigated.

2. Methods

2.1 Area of study

Three sampling cruises were mounted in the southern Black Sea in July 1997, March to April 1998 and September 1998. The station network for these cruises is illustrated in Figure 1. Hydrographic measurements were carried out on a grid and/or a transect system for determining the physical boundaries of the cyclonic and anticyclonic features and the sub-basin scale variations of the hydrography but these stations are not indicated in the network. The points for biochemical studies were selected by reference both to previous observations and to the real-time hydrographic data and the SeaWifs maps (for the Spring 1998 cruise). Previous studies showed that the physical oceanography of the Black Sea upper layer is dominated by the quasi-permanent cyclonic gyres in the eastern and western halves of the basin. The central gyres are separated from a series of small-scale anticyclonic eddies in the coastal zone by the cyclonically undulating rim current (Oğuz et al., 1991, 1993).

2.2 Sampling and analysis

The hydrographic data were collected using a Sea-Bird Model 9-11 CTD probe. In situ fluorescence data were collected continuously in the water column with a Chelsea-type fluorometer, attached to a CTD probe. A quantameter (LI-COR, LI-1000 Data Logger and LI-192SA Underwater Quantum Sensor) was used for irradiance (photosynthetically active radiation; PAR as $\mu E\ m^{-2} s^{-1}$) measurements in the water column. Water samples were collected with General Oceanic Go-Flo Rosette bottles attached to the CTD. The analyses of nutrient samples were carried out on board using a Technicon Model (new version, Bran Luebbe) two-channel autoanalyser. The colorimetric methods followed were similar to those described by Grasshoff et al. (1983). Chlorophyll-a (Chl-a) samples were collected from the euphotic zone down to the depths of <0.1% of surface light. Samples concentrated onto GF/F filters were extracted with 90% acetone solution. The fluorescence intensity of clear extracts was then measured using a Hitachi F-3000 Model spectrofluorometer. A commercially available Chl-a standard (Sigma) was used for calibration purposes (JGOFS, 1994). Dissolved oxygen (DO) and H_2S concentrations were determined by conventional Winkler and Iodometric Back Titration methods (Bastürk et al., 1994) while low H_2S concentrations were determined by the colorimetric method (Cline, 1969). The rates of carbon fixation by phytoplankton in the samples taken from the surface, 60, 36, 22, 8, 3, 1 and 0.1-<0.1% light depths were determined by a ^{14}C radio-tracing technique (Steemann-Nielsen, 1952), with slight modifications (Richardson, 1991). Incubations were performed both under artificial and sun light (simulated in situ) conditions. Bioassay experiments were performed by the addition of extra nutrients and initial concentrations were adjusted according to the maximum concentration levels observed in the deep water. Subsampling was regularly carried out (every 2-6 h) for subsequent analysis in order to determine the ambient nutrient concentrations. The growth efficiency of the phytoplankton was also checked by the addition of extra nutrients using the ^{14}C tracing technique.

3. Results

3.1 Hydrographic structure

The profiles of temperature (T), salinity (S) and density (sigma-theta, σ_t) from the southern Black Sea demonstrate that a nearly isohaline and relatively cool, isothermal water mass exists below the seasonal pycnocline (Figure 2). This prominent and persistent feature of the Black Sea, the CIL, possesses a temperature minimum which is characterised by the 8° C limiting isotherms (Oğuz et al., 1991). The σ_t of 14.8 isopycnal surface defines not only the temperature minimum within the CIL but also the upper boundary of the permanent pycnocline in the Black Sea (Murray et al., 1991). The thickness of the CIL was observed to be larger (65-120

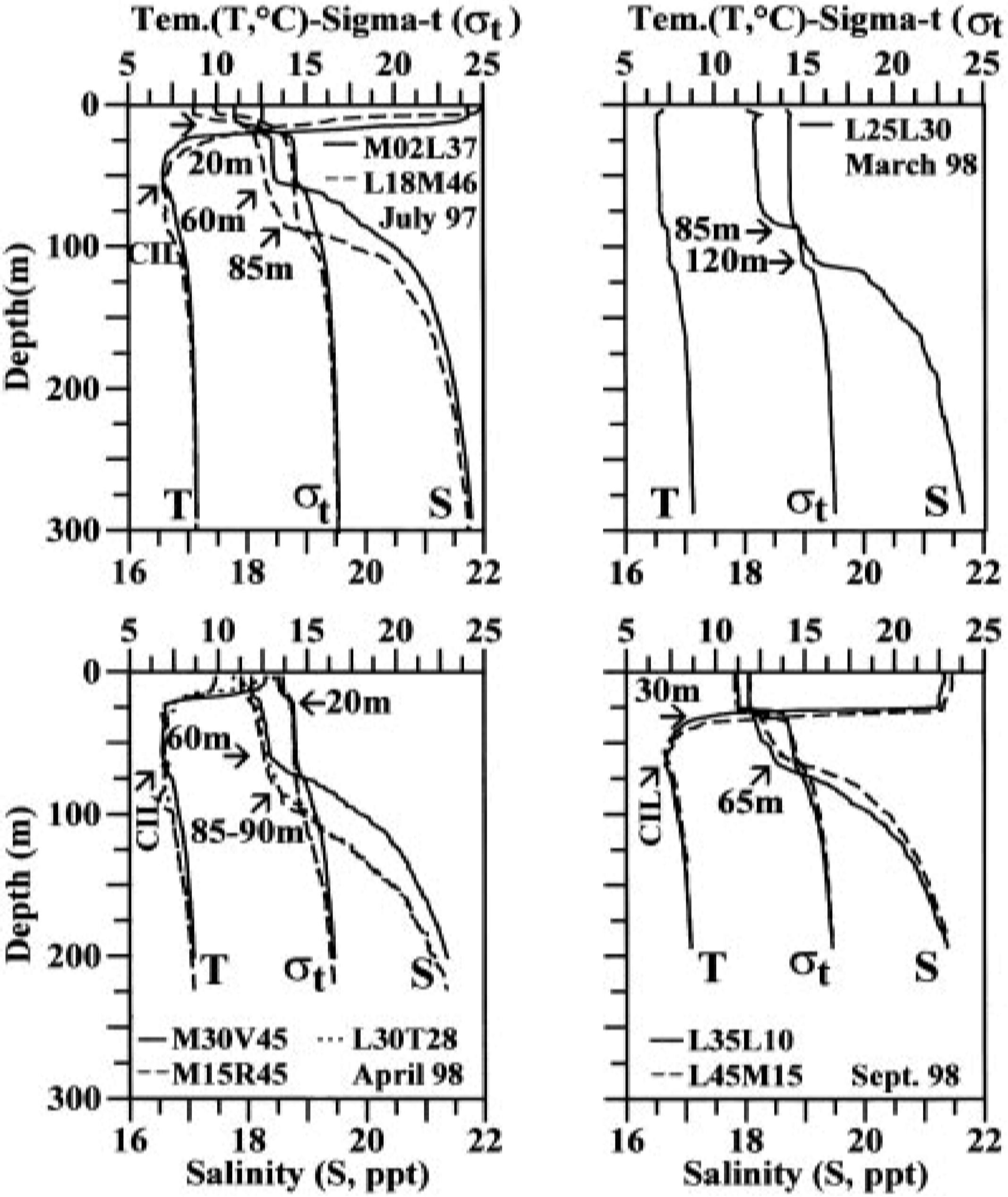


Figure 2 Vertical profiles of hydrographic parameters (unpublished data; with permission of Physical Oceanography Section, Institute of Marine Sciences, METU) in the southern Black Sea during July, 1997 – September, 1998 period.

m) in the nearshore region than in the cyclonic regions (60 m) and the salinity varied slightly within the range of 18 to 19 ppt in the CIL during the study period. In March 1998, when the surface waters cooled down to 7° C, the upper layer was throughly homogenised by convective mixing down to 120 m or down to the $\sigma_t = 14.8$ isopycnal surface (Figure 2). With the advent of heating in the early spring (April 1998), the surface temperature rose to 11-13° C and the CIL became topped by a warm surface layer. Below the CIL, the temperature gradu-

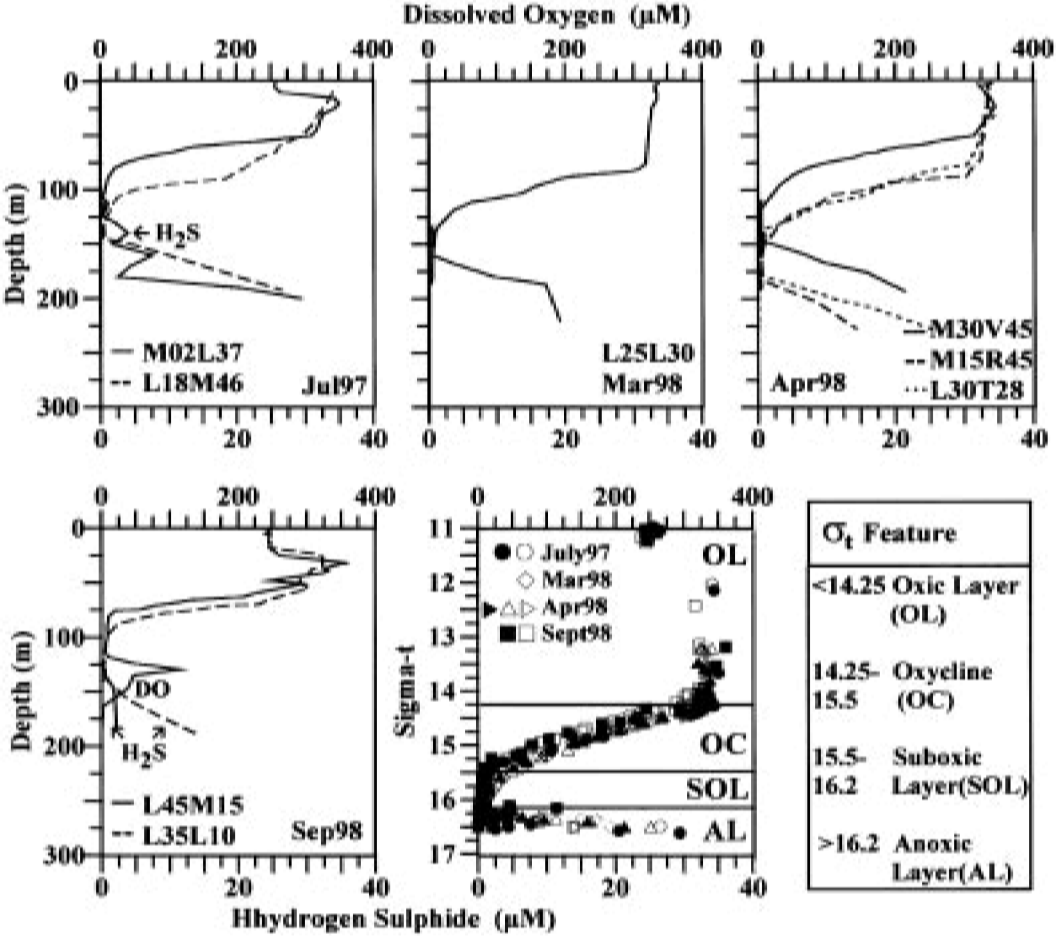


Figure 3 Vertical profiles of Dissolved Oxygen (DO, µM) and Hydrogen Sulphide (H_2S, µM). The curves in the upper part of the graph represent the DO profiles while the H_2S curves complete the profiles in the lower part. The profiles are also represented using the combined data and the sigma-theta as a vertical scale.

ally rose from 8° C to 8.7° C at the base of the permanent pycnocline; this was observed at different depths for different regions. The seasonal thermocline formed at 20 m in the late spring and it deepened (down to 30 m) in the autumn as clearly shown in the September 1998 example.

3.2 Chemical environment

Figure 3 shows that the surface layer down to the upper boundary of the temperature minimum in the CIL was nearly saturated with dissolved oxygen (DO; 250-350 µM). The concentrations decreased steeply in the upper depths of the permanent pycnocline from about 300 ± 50 µM at the σ_t =14.25 density surface to suboxic layer (SOL) concentrations of >20 µM at the σ_t =15.5. These density surfaces define the upper and lower boundaries of the main oxycline. As recently indicated (Basturk et al., 1997), the oxycline commenced at slightly greater density surfaces (s_t =14.4-14.5) but at shallower depths (50-75 m) in the cyclonic regions, than in the nearshore regions. The oxycline was located at s_t =14.2-14.3 (75-150 m) in the nearshore regions. Below the main oxycline, DO declined slowly to <5 µM at σ_t =15.9-16.0, and could no longer be detected at the σ_t =16.15-16.20 density surfaces where sulphide concentrations were 1-3 µM (Fig. 3). This DO-deficient water, formed within the oxic/anoxic transition layer with DO <20 µM and H_2S <1 µM, is the SOL. The lateral transport of the water masses is one of the well known features of the periph-

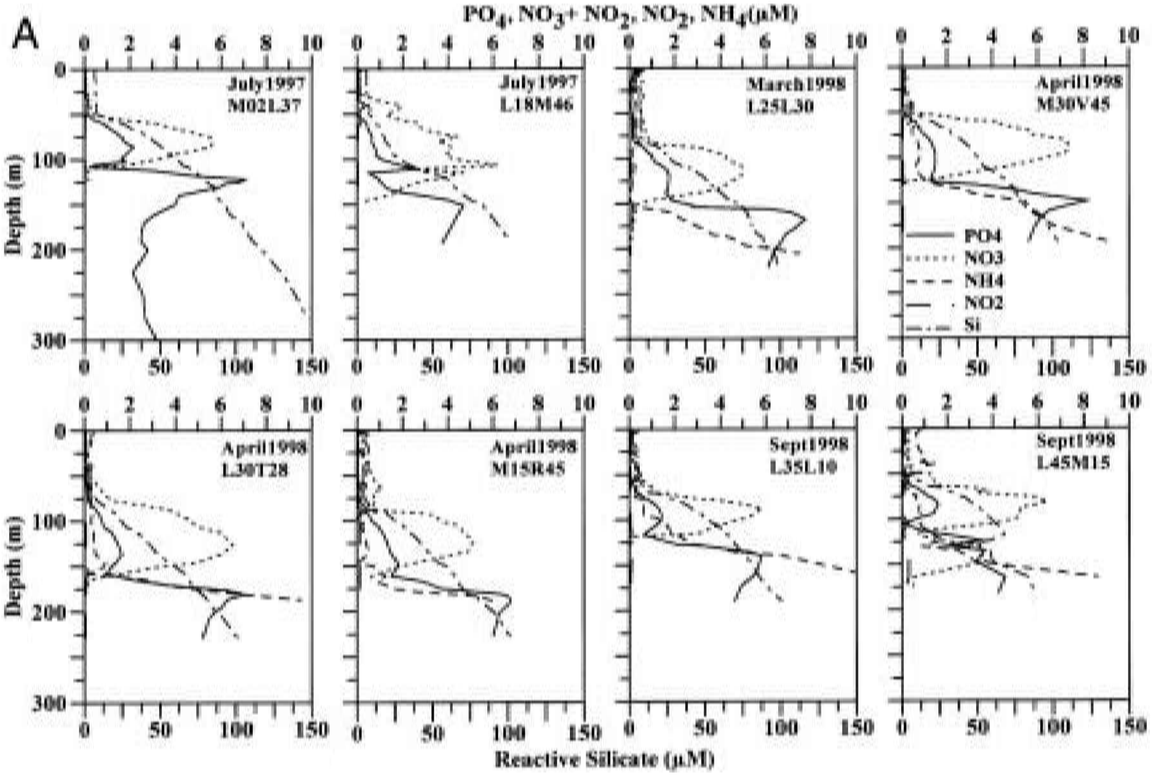
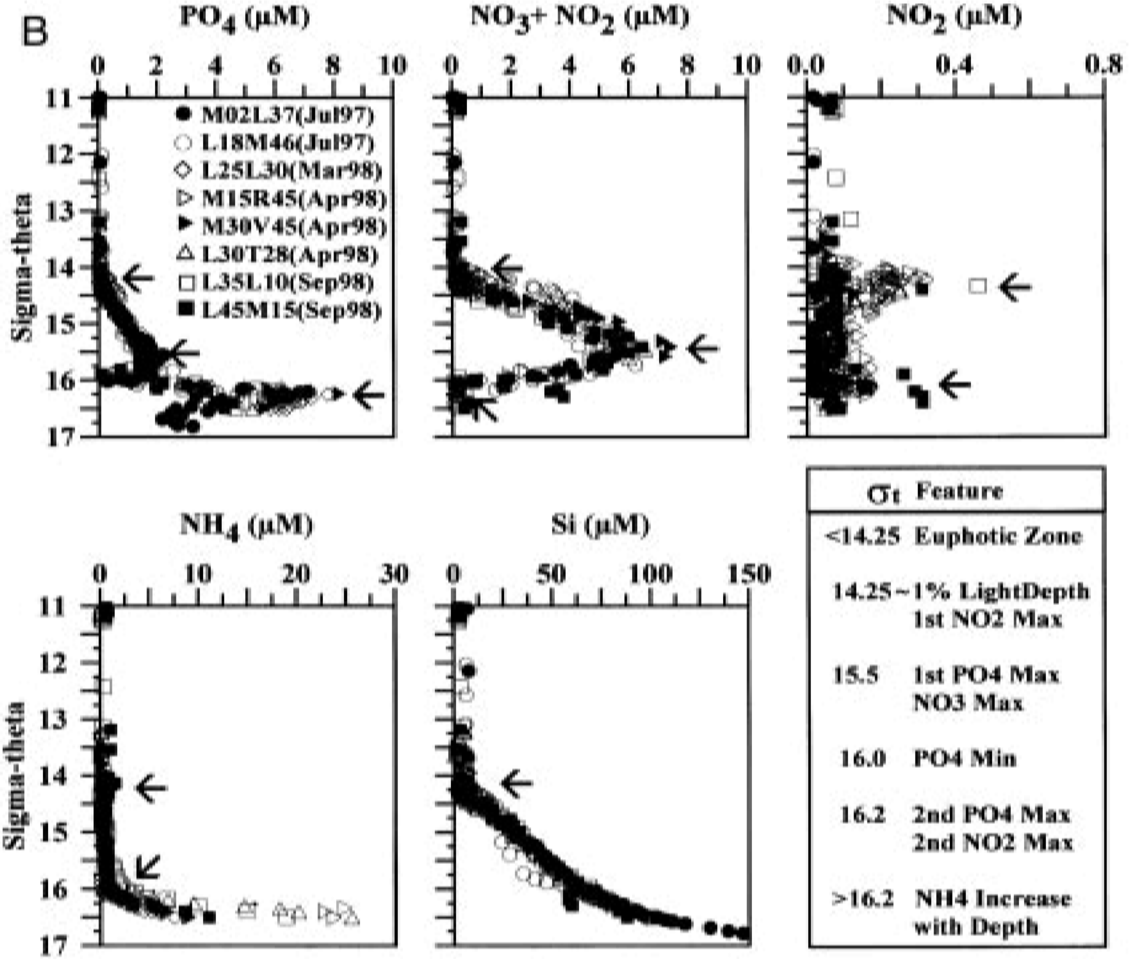


Figure 4a Depth profiles of dissolved nutrients: Ortho-phosphate (PO_4-P) Total Oxidised Nitrogen (NO_3 + $NO_2 \cong NO_3 - N$ in practice), Nitrite ($NO_2 - N$), Ammonium ($NH_4 - N$) and Reactive Silicate (Si).

eral areas near the rim current region (Oguz and Salihoglu, 2000) and the main hydrological structures related to the stratification are observed at shallower depths as in the cyclonic regions. As a consequence, the oxycline was located at shallower depths and below the SOL, sharp DO peaks (up to 75-100 µM) were observed in such regions (Figure 3, Stations M02L37-July 1997 and L45M15-Sept 1998). Sulphide-bearing waters were consistently observed at density surfaces of >16.15-16.2 over the entire basin. H_2S concentration increased steadily with depth, showing insignificant spatial or temporal variation at any density surface.

As previously emphasised (Basturk et al., 1994; Yilmaz et al., 1998b), the surface waters of the Black Sea are always poor in nutrients during the seasons of stratification. Concentrations in the euphotic zone were determined as <0.35 µM for PO_4, <0.5 µM for NO_3+NO_2, below detection limits for NH_4 and 5 µM for reactive silicate (Figure 4). The nutrient data from previous years (Yilmaz et al., 1998b) together with modelling studies (Oguz et al., 1996) indicate that intense vertical mixing in winter provides input from the nutricline which may increase surface nitrate concentrations to 5 to 10-fold. Composite profiles below the euphotic zone indicated that nutrient concentrations increased with increasing density down to the base of the main oxycline (Figure 4b and Figure 3). The nitrate concentrations displayed a well-defined maximum (up to 7.5 µM) at 15.5 density surface defining the upper boundary of the SOL, where, due to the denitrification processes, nitrate concentrations declined steadily to 0.1-0.2 µM at the suboxic/anoxic interface. The nitrite concentration was always observed to be less than 0.5 µM and two maxima were observed at σ_t = 14.25 and σ_t = 16.2 density surfaces due to the nitrification and denitrification processes, respectively. The NH_4 concentration was always below detection limit in the euphotic zone and this feature was prominent down to σ_t = 16.2 isopycnal surface; below this depth NH_4 concentration increased steadily with depth. Phosphate concentrations increased within the oxycline to a maximum in the upper SOL or at around σ_t = 15.5 isopycnal surface coinciding with the NO_3 maxi-



σt	Feature
<14.25	Euphotic Zone
14.25~	1% LightDepth 1st NO2 Max
15.5	1st PO4 Max NO3 Max
16.0	PO4 Min
16.2	2nd PO4 Max 2nd NO2 Max
>16.2	NH4 Increase with Depth

Figure 4b Profiles of dissolved nutrients: Sigma-theta (σ_t) was used instead of depth on the vertical scale.

mum. Below this broad maximum (the first max), it declined steeply in the cyclonic regions, forming a pronounced minimum (0.05-0.10 μM) at the σ_t = 16.0 isopycnal surface. This feature is less marked in the nearshore region (Figure 4). Nevertheless, PO_4 concentrations always increase steeply within the sulphidic water interface and reach peak values of 6-8 μM (the second max) at σ_t = 16.2 isopycnal surface. The PO_4 concentration decreased slightly in the upper AL and then increased again slightly with depth. The occurrence of a marked maximum at the sulphidic boundary results from dissolution of PO_4 associated Fe- and Mn-oxides in the anoxic waters (Shaffer, 1986; Codispoti et al., 1991). Silicate concentration increased steadily below the euphotic zone or below σ_t = 14.25 density surface with increasing depth and density.

3.3 Primary productivity and related features

Chlorophyll-a and in situ fluorescence (RF, as Relative Fluorescence) data are shown in Figure 5. The Chl-a concentrations in the euphotic zone were generally low (<0.5 μg l^{-1}) if one excludes the March 1998 example. A subsurface Chl-a maximum having a concentration value up to 1.5 μg l^{-1} as well as the RF maximum formed near the base of the euphotic zone (>0.1 - <1% light depth) and below the seasonal thermocline or at a certain density surface, σ_t = 14.2 ± 0.05 (Figure 5). In the central cyclonic gyres where the upper boundary of nutricline is shallower (Figure 4a), the subsurface maxima were followed by a significant decrease while the Chl-a profiles (as well as in situ RF) have shown a secondary deep maximum in the nearshore region. The deep sec-

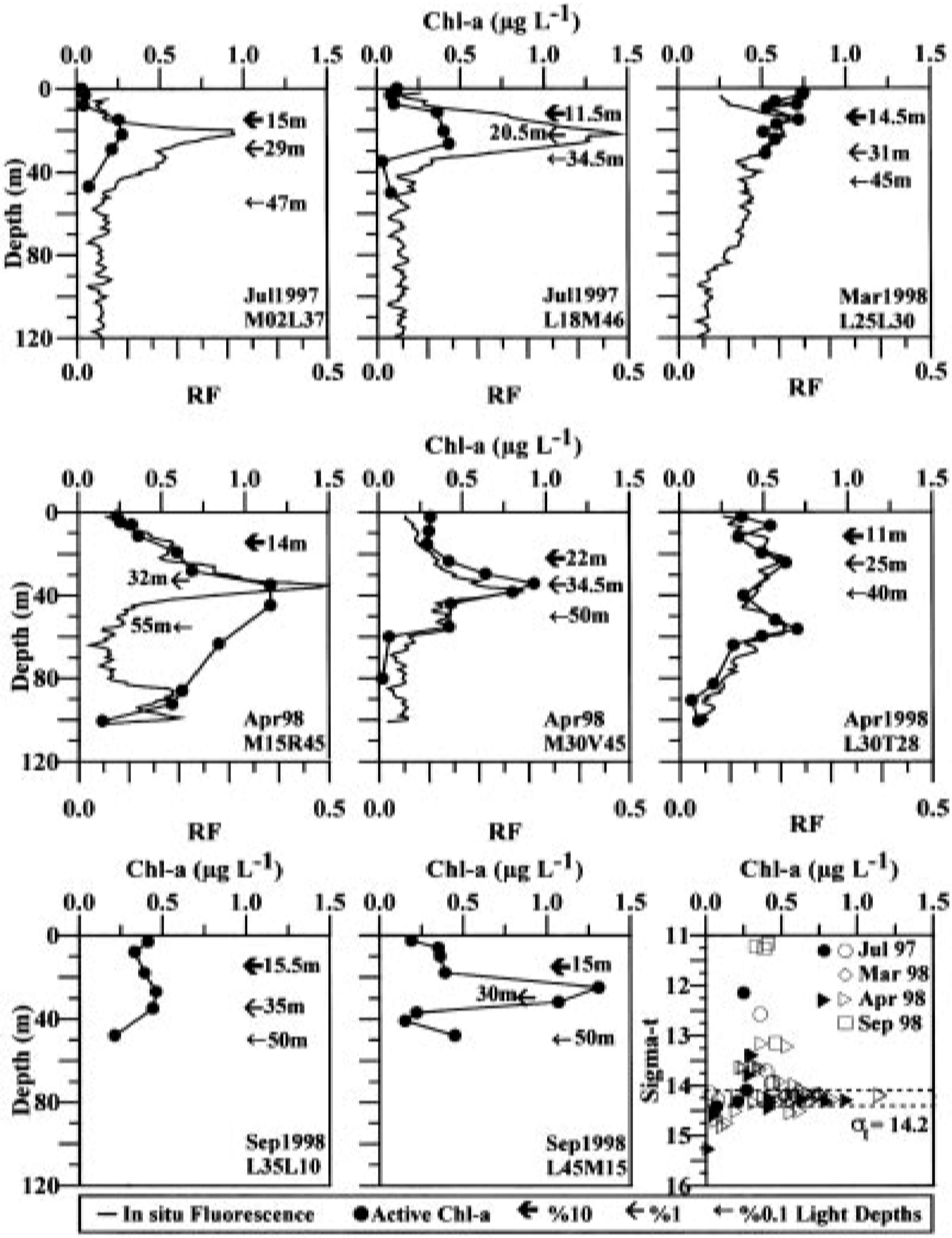


Figure 5 Vertical profiles of Chlorophyll-a (Chl-a, µg l^{-1}) and in situ fluorescence (as relative fluorescence intensity: RF) for the stations in the southern Black Sea visited during July 1997 to September 1998 period. Default symbols represent the Chl-a concentrations of discrete samples while the continuous profiles (Solid line) represent in situ fluorescence intensity due to Chl-a. 10%, 1% and 0.1% (mostly determined by extrapolation) light depths were marked by arrows. At the last graph Sigma-theta (σ_t) was used as the vertical scale and all the Chl-a data were combined.

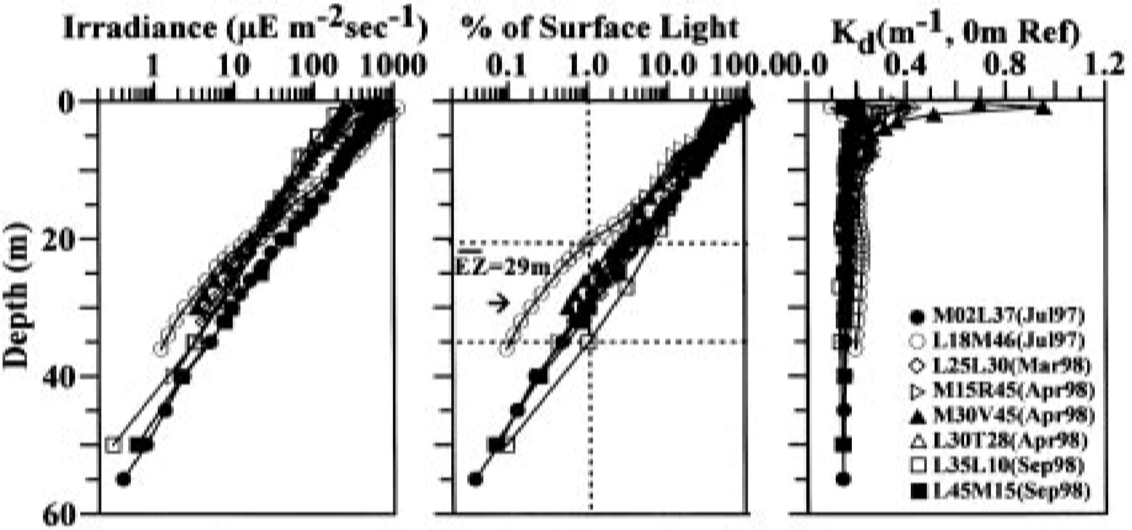


Figure 6 Penetration of light as downward irradiance, I, (µE m^{-2} s^{-1}); as the percentage of surface light (% of Io). Downward attenuation coefficient (K_d, m^{-1}) were calculated using the surface irradiance as a reference value.

ondary maxima were located at very low level of light (at 0.1-0.3 µE $m^{-2}s^{-1}$ or at 0.01-0.1% light depths). In March 1998, Chl-a showed almost uniform vertical distribution within the range of 0.7-1.0 µg l^{-1}.

The thickness of the euphotic zone (practically defined as the depth of 1% of the surface light) ranged between 20 and 35 m in the southern Black Sea for the study period (Figure 6). The 0.1% light depth was in the range of 35 to 50 m and this layer also seems to be the photosynthetically active layer in the Black Sea as previously recorded (Vedernikov and Demidov, 1997; Yilmaz et al., 1998b) and thus the lower boundary of the euphotic zone could be considered in the range of >0.1 to <1% light depths. The less energetic, high wavelength component of the incoming light was absorbed in the upper surface layer (the top 5 m), where the highest (downward) attenuation coefficient (K_d = 0.2-1.0 m^{-1}) was calculated. Below this layer the solar light penetrated with a constant K_d, which varied regionally between 0.1 and 0.25 m^{-1}. The highest estimated K_d values were observed in the nearshore region where the phytoplankton biomass and the photosynthetic production rates were relatively high.

The primary productivity rates varied between 1 and 6 mg C m^{-3} h^{-1} and the highest rates were always determined in the upper euphotic zone down to the 10% light depth or the top 10 to 15 m of the water column. Below this layer, the rate decreased markedly with depth and dropped to insignificant rates at the 1% light depth (Figure 7a). The rates were relatively high at low light levels below the euphotic zone in the nearshore regions. In order to estimate the capacity of potential production of the phytoplankton populations, samples taken from different light depths were exposed to the full artificial light conditions. The estimated maximum rates from low light levels (0.1-<0.01% of the surface light), were comparable with the surface values and the subsurface maxima of photosynthetic production rates coincided with the subsurface Chl-a and RF maxima (Figures 5, 7a). The secondary but relatively weak peak of photosynthetic carbon production rates was observed at the same depths with deep secondary RF maxima (as deep as 70-100 m) in the nearshore region (See April 1998 profiles). The population living here was determined to be mostly diatoms (E. Eker, Middle East Technical University, Turkey, unpubl. data) and though a very low level of in situ light, (<0.5 µE m^{-2} s^{-1}) was present, they were capable of photosynthesis when that light was available. Such deep secondary maxima were not observed in the central cyclones in the study period. The depth-integrated production rates are presented in Table 1. The highest rates were recorded (601-785 mg C $m^{-2}d^{-1}$) in the seasons of well stratification. These values are still lower than the values already known for the northwestern shelf and off the Romanian coast for the 1970-1980 period (Bologa, 1985/1986) and were very similar to those of the central Black Sea given for the late 1980 and early 1990s (Vedernikov and Demidov, 1993). Paral-

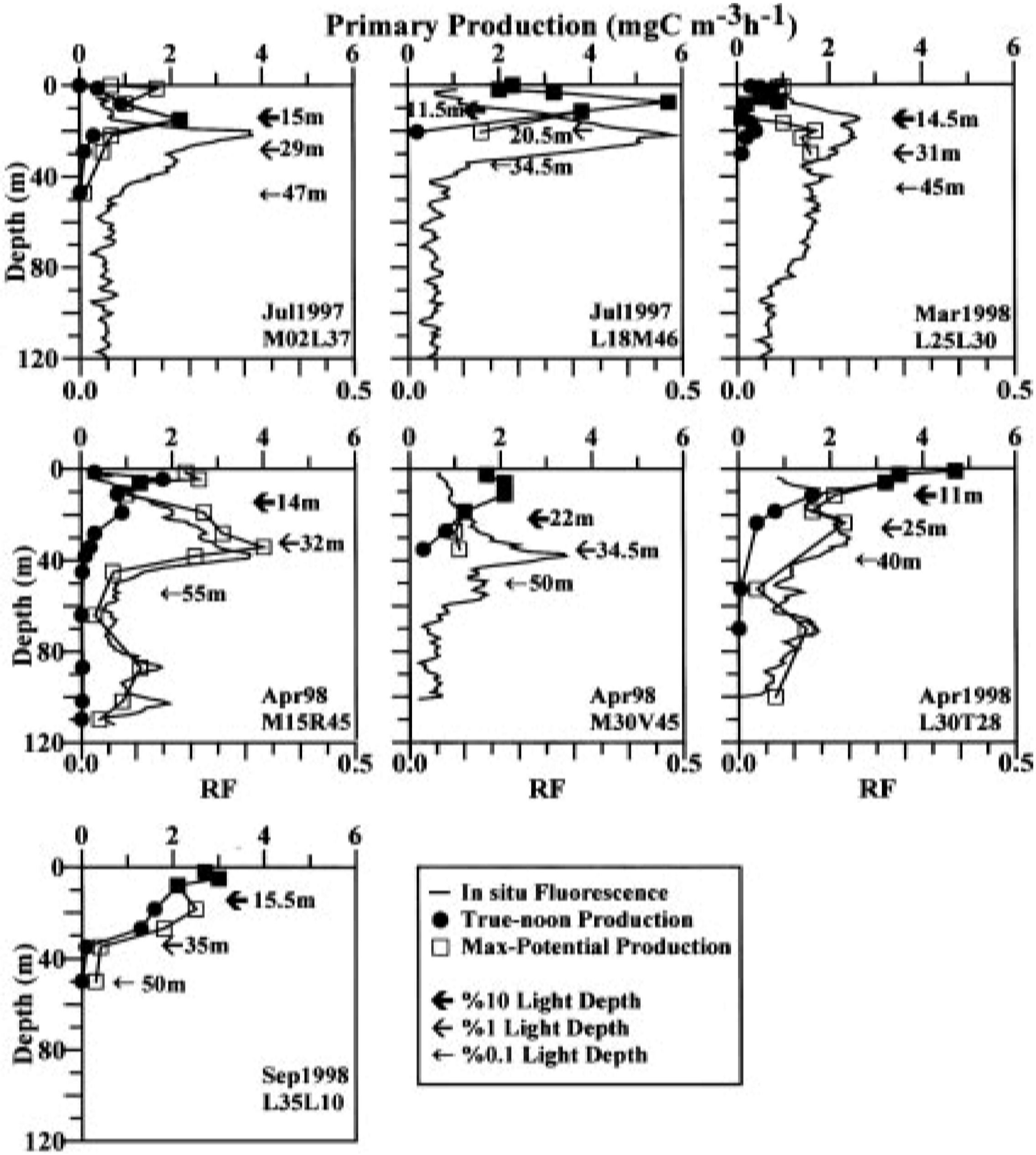


Figure 7a Vertical profiles of total primary production and maximum potential production rates (mg C m^{-3} h^{-1}) estimated for the Black Sea using artificial light conditions and a constant temperature water bath (artificial incubator). Continuous lines represent the in situ fluorescence (RF) profiles.

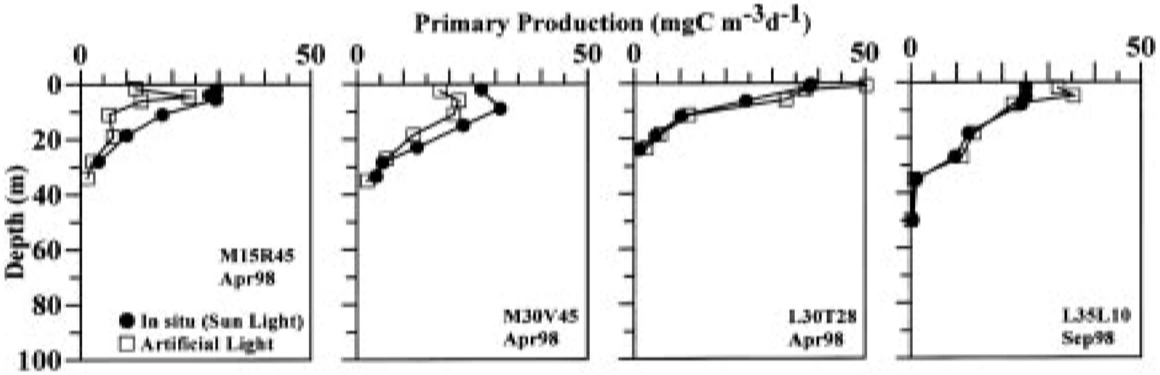


Figure 7b Daily production rates (mg C m^{-3} d^{-1}) estimated using simulated in situ and artificial incubation conditions. Light source was sunlight and the constant temperature was provided by pumping the surface seawater into the Plexiglas incubators on the deck during simulated in situ experiments.

lel samples were incubated simultaneously under sunlight and generally full day exposures were performed during April 1998 and September 1998 cruises (Figure 7b). The daily integrated production rates were estimated in the range of 420-639 mg C m^{-2} d^{-1} for this period which were comparable to the values estimated for the samples incubated under artificial light conditions (Table 1). The differences between two incubation methods could be attributable to the differences in the light sources and the incubation temperature. In the case of artificial light incubation under fluorescence lamps, the temperature of the incubator was adjusted to the temperature of the 50% light depth while the surface water was pumped to the Plexiglas pools where the simulated in situ experiments were performed.

Bioassay experiments, using extra PO_4, NO_3, NH_4, Si and Fe were performed during the present study. The data showed that under optimum light conditions the phytoplankton population is NO_3 limited in the central cyclonic region (Station M30V45) and the peripheral area between the central cyclone and the rim current (Station L45M15) (Figure 8). If the nutrients are separately added to the samples, the decrease in the ambient NO_3 concentration was not as significant as the trend observed for the medium which was enriched with all nutrients (Cocktail: PO_4, NO_3, NH_4, Si and Fe). While the NO_3 was efficiently used in the open waters, PO_4 concentration remained almost constant during the 2 to 3 d incubation. Phosphate seems to control the growth in the nearshore regions of the Black Sea (Stations L18M46-July, 1997 and M15R45-April, 1998) and the decrease of ambient PO_4 concentration was clearly observed during 1 to 2 d incubation (Figure 8). In July 1997, the adaptation of the phytoplankton population

Table 1 Daily integrated production rates (DIPR) (mg C m^{-2} d^{-1}) calculated down to 1% light depth or for the euphotic zone (EZ) in the Black Sea.

Date	Station	Location	EZ (m)	DIPR (Artificial Light)	DIPR (Simulated In Situ)
Jul97	M02L37	Deep(W)	29	373	-
Jul97	L18M46	Nearshore(W)	20.5	785	-
Mar98	L25L30	Nearshore(W)	31	62	-
Apr98	M15R45	Nearshore (E)	32	257	461
Apr98	M30V45	Deep (E)	34.5	461	639
Apr98	L30T28	Nearshore(E)	25	447	420
Sep98	L35L10	Nearshore(W)	35	601	546
	Average	**The Black Sea**	**29.6 ± 5.2**	**487 ± 184**	**516 ± 97**

* L25L30 was excluded during calculation of averages and standard deviation

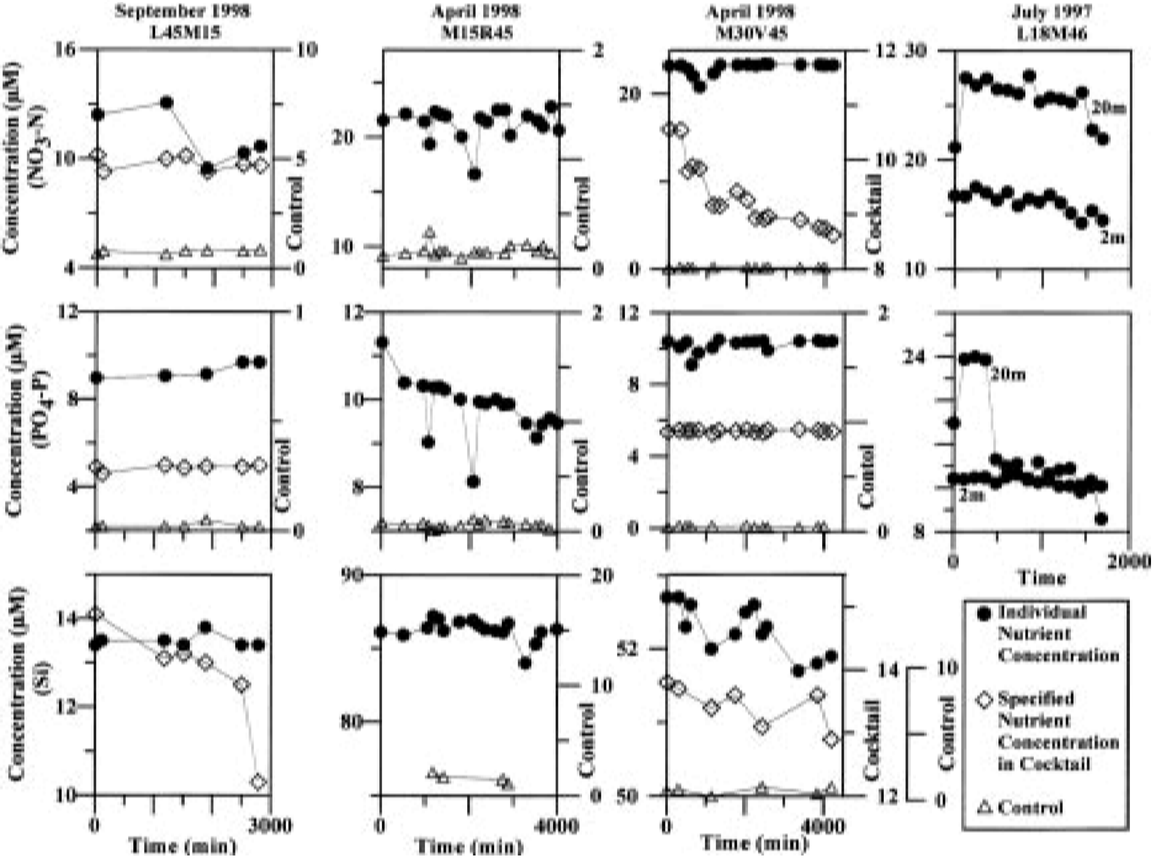
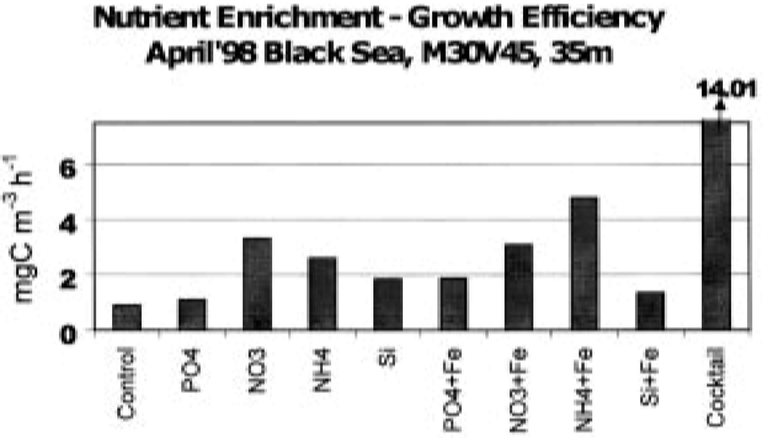


Figure 8 Nutrient addition experiments performed during July 1997, April 1998 and September 1998 cruises. The nutrients (PO_4, NO_3 and Si) were separately added and the initial concentrations (10-30 µM, 4-18 µM and 10-90 µM for phosphate, nitrate and silicate respectively) were adjusted according to highest concentrations in the deep water. Cocktails were prepared using all nutrients (including Fe) and only the concentration changes of PO_4, NO_3 and Si were determined.

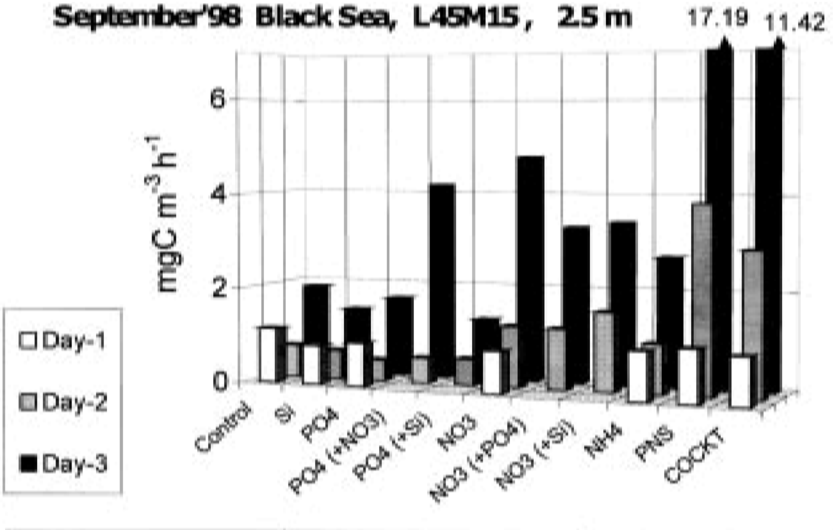
to higher nutrient concentration levels at the beginning of the incubation was well established. The phytoplankton populations sampled from low light levels (from 1% light depth; 20 m) indicated more efficient uptake of PO_4 when compared with the population living at the surface where the in situ RF and the Chl-a maxima were observed (Figure 5). The uptake of NO_3 was not as significant as the PO_4 for the same population. In the case of Si addition (Figure 8), phytoplankton growth should be affected by the ambient Si concentration since the majority of the population was determined to be diatom at all stations in the Black Sea for April 1998 and September 1998 periods (E. Eker, Middle East Technical University, Turkey, pers. comm.). The Si uptake occurred in samples collected from the two deep stations (M30V45 and L45M15). At station L45M15, the Si concentration diminished in the cocktail bottle, but not in the solely Si-enriched bottle. On the other hand, at station M30V45, the decrease in Si concentration was detected in both Si-enriched and cocktail bottles. In samples from station M15R45, no Si uptake was detected.

Nutrient enrichment bioassays were also performed in order to test the growth efficiency using the ^{14}C technique. During April 1998 cruise, the bioassays could be completed at station M30V45 in the central cyclone (Figure 9). The water sample collected from 1% light depth (corresponding to the in situ RF and the Chl-a maxima at 35 m) was enriched with each nutrient, nutrients plus Fe, and with all nutrients and Fe (Figure 9; see legend for the enrichment concentrations). The results showed that the growth was stimulated by the addition of NO_3 and NH_4. The efficiency of uptake and subsequent assimilation of NH_4 was more significant when it was introduced to the system with Fe. On the other hand,



Sample	Nutrient Enrichment (μM)
Control	—
PO_4	10
NO_3	20
NH_4	5
Si	50

Sample	Nutrient Enrichment (μM)
PO_4 + Fe	10 + 0.2
NO_3 + Fe	20 + 0.2
NH_4 + Fe	5 + 0.2
Si + Fe	10 + 0.2
Cocktail	$5PO_4 + 10NO_3 + 4NH_4$+15Si+ 0.2 Fe



Sample	Enrichment(μM)	Sample	Enrichment (μM)	Sample	Enrichment (μM)
Control	—	PO_4(+Si)	5 (+16)	NH_4	4
Si	10	NO_3	10	PNS	5 PO_4+10NO_3+10 Si+ 2 NH_4
PO4	5	NO_3 (+PO_4)	10 (+6)	COCKT	(PNS) + 0.2 Fe+EDTA
PO_4(+NO_3)	5 (+8)	NO_3(+Si)	10 (+16)		

Figure 9 Growth efficiency of phytoplankton populations collected from the eastern cyclone (Station M30V45) and from peripheral area (Station L45M15) in the western Black Sea. The sampling depths are 35m (1% of the surface light) 2.5m respectively. At station M30V45, the nutrients (PO_4, NO_3, NH_4 and Si) were added separately, as nutrient plus iron and all together (Cocktail). After the addition of nutrients and 2 days incubation under the sunlight the appropriate and equal amount of ^{14}C radiotracer were added to each sample and they were exposed to full artificial light conditions and carbon uptake rates were determined as mg C m^{-3} h^{-1}. At station L45M15, the water samples were exposed to sunlight after the addition of nutrients (PO_4, NO_3, NH_4 and Si) separately, and all together (PNS: PO_4, NO_3, NH_4 and Si; Cocktail: PO_4, NO_3, NH_4, Fe and Si). Then the subsamples were exposed to full artificial light conditions at each day by the addition of equal amount of ^{14}C radiotracer. Extra subsamples were prepared for different combinations of nutrient pairs for the second and third days' tests. Nutrient enrichment concentrations are separately indicated in the legends.

when all nutrients were available, the growth efficiency increased significantly, providing almost 15 times higher uptake rates. The stimulation of ^{14}C uptake with Si and PO_4 enrichment was not as significant as that with NO_3 or NH_4 enrichment.

Similar but more detailed experiments were performed during the September 1998 cruise in the peripheral area, at Station L45M15 (Figure 9). The water sample was collected from 60% light depth (2.5m) and seven transparent Pet bottles (5l each) were incubated under sunlight (Day 1). The samples consisted of a control sample with no extra nutrient addition, four bottles enriched with PO_4, NO_3, NH_4 and Si separately, a Cocktail bottle enriched with all four major nutrients and excessive Fe, and a bottle enriched with all of the four major nutrients but without extra Fe addition (PNS; See the legend in Figure 9b for the enrichment concentrations). During the 3 d incubation, the growth efficiency corresponding to each bottle was tested on the sub-samples each day using ^{14}C tracing technique. The results generally indicated that additions of NO_3 and NH_4 stimulated carbon uptake, supporting the statement that the deep waters of the Black Sea are N-limited. Phosphate and Si enrichment were slightly inhibitory. As expected, when all nutrients (including Fe) were available for the phytoplankton population, the growth efficiency increased considerably (Figure 9). Comparison of the results obtained from PNS and Cocktail subsamples indicated that stimulation was relatively less pronounced with the Fe addition. It is should be noted here that the final Fe concentration was as high as 0.2 µM which may have been too high for the natural growth of phytoplankton population, that is, it may have acted as an inhibitor.

To check whether the nutrient enrichment leads to an adaptive change in nutrient preferences of the sampled population, at the second day of the experiment, an additional subsample taken from the NO_3 enriched bottle was enriched again with PO_4 and this subsample was denoted as NO_3 (+PO_4), and subjected to ^{14}C incubation. The result showed that the PO_4 addition to this subsample did not stimulate C-uptake, on the contrary, it decreased slightly as compared to the non-enriched subsample from the same bottle (Figure 9). A similar procedure was carried out with another subsample from the NO_3 bottle; enriched with Si and denoted as NO_3 (+Si). The immediate effect of Si addition was a relative increase in the C uptake rate when compared with the non-enriched subsample. A subsample taken from the PO_4 bottle was enriched with $NO_{3,}$ and denoted by PO_4 (+NO_3). The ^{14}C incubation of this subsample showed that the addition of NO_3 increased the C-uptake rate slightly, in comparison to the non-enriched subsample. Another subsample from the PO_4 enriched bottle was enriched by Si; in turn, it was observed that the production rate increased slightly. Exactly the same procedure was followed at the third day of the experiment (Figure 9). The addition of PO_4 to the subsample taken from the NO_3 enriched bottle immediately caused a pronounced decrease in C-uptake. Similarly in the NO_3 (+Si) subsample, the addition of Si has resulted in a decrease in the primary production rate. On the contrary, addition of NO_3 to the subsamples taken from the phosphate enriched bottle, caused more than two-fold increase in the primary production rate during third day experiment, relative to the non-enriched PO_4 subsample. Si addition to previously PO_4 enriched sample caused a decrease in the production rate.

4. Discussion

Primary production rates in the Black Sea determined in the present study are similar to those reported for the open part of the north-western shelf and off the Romanian coast (Bologa, 1985/1986) and exceed those observed in the central Black Sea reported for the period before 1990 (Vedernikov and Demidov, 1993). The highest rates (e.g., up to 785 mg C m^{-2} d^{-1} in July 1997) were observed in the nearshore areas and/or in the rim current region in the western Black Sea. Chlorophyll-a concentrations showed a different trend; there was no significant difference between nearshore and deep regions in terms of sub-surface maximum concentrations and integrated Chl-a concentrations (determined as 0.68-0.84 µg l^{-1} and about 14.2 mg m^{-2}, respectively). On the other hand, the average surface Chl-a concentration at the nearshore stations was double the average value obtained for the central deep stations. Relatively high primary production rates obtained during stratification seasons (July 1997 and September 1998) are consistent with the occurrence of short summer blooms in the Black Sea additional to the characteristic spring bloom (Bologa, 1985/1986; Hay et al., 1990; Vedernikov and Demidov, 1993; Yilmaz et al., 1998a, 1998b). Such summer and early autumn blooms are mainly dominated by coccolithophores as reported by the same authors and the microscopic analysis of the samples collected for the present study showed a similar trend (29 and 72% of the total phytoplankton abundances were coccolithophores while the biomass were dominated by diatoms for July, 1997 and September, 1998 respectively) (Z. Uysal and E. Eker, Middle East Technical University, Turkey, pers. comm.). During spring 1998, the rates of primary production were not high (62-461 mg C m^{-2} d^{-1}) as expected and as reported (up to 1,200 mg C m^{-2} d^{-1} for the deep regions of the Black Sea for February-March, 1961-90 period; Vedernikov and Demidov, 1993). In general, the prominent sub-surface Chl-a maxima as well as the potential production maxima were formed at the base of the euphotic zone (~ at around 1% light depth and $\sigma_t = 14.2$ density surface) in the Black Sea. Secondary Chl-a maxima were encountered below the euphotic zone at two stations (M15R45 and L30T28) in the nearshore region during the April 1998 cruise. The deep secondary maxima (the so-called sub-euphotic Chl-a maxima) were located at very low level of light (at 0.1-0.3 μE m^{-2} s^{-1} or at 0.1-0.01% light depth).

The ^{14}C experiments carried out on the samples collected from these sub-euphotic Chl-a maxima showed that the phytoplankton were able to perform photosynthesis in significant amounts when exposed to substantial irradiances in the artificial incubator. The microscopic analysis for taxonomic distribution revealed that the diatom *Rhizosolenia* sp., especially *Rhizosolenia alata*, were present in significant abundance in the water column during this period and always dominated in terms of biomass at the depths below the euphotic zone, for example, at the sub-euphotic maxima (E. Eker, Middle East Technical University, Turkey, pers. comm.). Rhizosolenia species are known to migrate vertically which enable them to exploit nutrient sources of deeper waters (Villareal and Carpenter, 1994; Villareal and Lipschultz, 1995, cited in Richardson et al., 1998; Moore and Villareal, 1996). There is clearly a lack of data and of information on the *Rhizosolenia* migrations in the Black Sea. Further investigations are obviously necessary in order to understand the importance of such processes, especially the role in new production due to import of new N from the nutricline in the Black Sea.

Bioassay experiments indicated PO_4 limitation in the nearshore regions, while deeper regions appeared to be N-limited in terms of primary production in the Black Sea. The bioassay results are consistent with the molar NO_3/PO_4 ratios of surface waters. Whereas anthropogenic impact (increase in N and P loads) causes an increase in N and P concentrations in the Black Sea, Si input has declined during the last decades (Cociasu et al., 1996; Humborg et al., 1997). The depletion of Si was attributed to the construction of the Iron Gates dam on Danube river in the early 1970s. These processes resulted in dramatic shifts in phytoplankton species compositions and the cell abundances from siliceous (mainly diatoms) to non-siliceous species, mainly to coccolithophores and flagellates (Cociasu et al., 1996; Humborg et al., 1997). The nutrient addition bioassays performed in the present study indicated Si shortage both in the eastern and western deep stations. In the station M30V45, where phytoplankton exhibited highest tendency for Si-uptake, the size fractionated production experiments indicated a decreased share of large cells in the production. The microscopic analysis (Elif Eker, Middle East Technical University, Turkey, pers. comm.) indicated that the larger cells in the Black Sea are usually diatoms, which need significant amounts of dissolved Si for their growth.

One should be very cautious in confirming that nutrient limitation has occurred based on the results of nutrient enrichment bioassays, especially with the ^{14}C method. It can be stated that these essays usually give a fair estimation regarding the limiting nutrient, but it is conjectural to suggest that they reflect the uptake kinetics of the populations in their natural environment. Due to sampling, incubation and especially nutrient enrichment processes, the population dynamics in vitro are definitely different from in situ conditions. The basic hypothesis in the determination of limiting nutrient by the ^{14}C method is that a rapid increase in the photosynthetic rate will occur following the addition of the nutrient. However, Healey (1973, 1979, cited in Lean and Pick, 1981) argues that a lag in the increase in photosynthetic rate is more likely and shows that the metabolism of the algae first tends towards nutrient uptake processes, than to the conversion of previously stored C to cell constituents for growth. Lean and Pick (1981) performed C uptake measurements by lake plankton at different levels of added P. They reported that nutrient deficient populations seemed to temporarily decrease, rather than enhance photosynthesis when P became available. The results of ^{14}C uptake measurements on the nutrient enriched samples performed in the present study are in agreement with Healey's suggestion in the case of P. The addition of PO_4 to P-limited samples (incubated in NO_3 enriched media for 2 -3 d) resulted in an immediate decrease in the ^{14}C uptake rate, also in accordance with the behaviour reported by Lean and Pick (1981). In the case of N, however, it has been found that the addition of NO_3 to the N-limited populations resulted in an immediate increase in the photosynthetic rate. Although the data acquired in the present study are not decisive, it is quite probable that the metabolic interactions between PO_4 and C uptake processes are quite different from the interactions of NO_3 and C-uptake in the algal cells.

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References

Basturk, O., Saydam, C., Salihoglu, I., Eremeev, L.V., Konovalov, S.K., Stoyanov, A., Dimitrov, A., Cociasu, A., Dorogan, L., Altabet, M., 1994. Vertical variations in the principal chemical properties of the Black Sea in the autumn of 1991. Mar. Chem. 45, 149-165.

Basturk, O., Tugrul, S., Konovalov, S., Salihoglu, I., 1997. Variations in the vertical structure of water chemistry within the three hydrodynamically different regions of the Black Sea. In: Özsoy, E., Mikaelyan, A. (Eds.), Sensitivity to Change: Black Sea, Baltic Sea and North Sea, NATO ASI Series, 27, 183-196. Kluwer Academic Publishers, the Netherlands.

Bologa, A.S., 1985/86. Planktonic primary productivity of the Black Sea: a review. Thalassia Jugoslavica 21/22(1/2), 1-22.

Bologa, A.S., Bodeanu, N., Petran, A., Tiganus, V., Zaitsev, Y.P., 1995. Major modifications of the Black Sea benthic and planktonic biota in the last three decades. Bull. l'Instit. Oceanogr., Monaco Special 15, 85-110.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulphide in natural waters. Limnol. Oceanogr. 14, 454-458.

Cociasu, A., Dorogan, L., Humborg, C., Popa, L., 1996. Long-term ecological changes in Romanian Coastal Waters of the Black Sea. Mar. Poll. Bull. 32, 32-38.

Cociasu, A., Diaconu, V., Popa, L., Nae, I., Buga, L., Dorogan, L., Malciu, V., 1997. Nutrient stock of the Romanian shelf of the Black Sea in the last three decades. In: E. Özsoy, A. Mikaelyan (Eds.), Sensitivity to change: Black Sea, Baltic and North Sea. NATO ASI Series, 27, 49-63. Kluwer Academic Publishers, the Netherlands.

Codispoti, L.A., Friederich, G.E., Murray, J.W., Sakamato, C.M., 1991. Chemical variability in the Black Sea: Implications of continuous vertical profiles that penetrated the oxic/anoxic interface. Deep Sea Res. 38, 691-710.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Determination of nutrients. Methods of SeaWater Analysis. 2nd edition, pp. 125-188. Verlag Chemie GmbH, Weinheim.

Hay, B.J., Honjo, S., Kempe, S., Itekkot, V.A., Degens, E.T., Konuk, T., Izdar, E., 1990. Interannual variability in particle flux in the southwestern Black Sea. Deep Sea Res. 37, 911-928.

Humborg, C., Ittekot, V., Cociasu, A., Bodungen, B.V., 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. Nature 386, 385-388.

JGOFS (Joint Global Ocean Flux Study), 1994. Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements, Manual and Guides. 29, 97-100.

Lean, D.R.S., Pick, F.R., 1981. Photosynthetic response of lake plankton to nutrient enrichment: A test for nutrient limitation. Limnol. Oceanogr. 26(6), 1001-1019.

Mee, L.D., 1992. The Black Sea in crisis: The need for concerted international action. Ambio 21, 278-286.

Moore, J.K., Villareal, T.A., 1996. Size-ascent rate relationships in positively buoyant marine diatoms. Limnol. Oceanogr. 41(7), 1514-1520.

Murray, J.M., Jannasch, H.W., Honjo, S., Anderson, R.F., Reeburgh, W.S., Top, Z., Friederich, G.E., Codispoti, L.A., Izdar, E., 1989. Unexpected changes in the oxic/anoxic interface in the Black Sea. Nature 338, 411-413.

Murray, J.M., Top, Z., Ozsoy, E., 1991. Hydrographic properties and ventilation of the Black Sea. Deep Sea Res. 38, S663-S690.

Murray, J.M., Codispoti, L.A., Freiderich, G.E., 1995. Oxidation-reduction environments: The suboxic zone in the Black Sea. In: C.P. Huang, C.R. O'Melia, J.J. Morgan (Eds.), Aquatic Chemistry, ACS Advances in Chemistry Series 244, 157-176.

Oguz, T., Salihoglu, B., 2000. Simulation of eddy-driven phytoplankton production in the Black Sea. Submitted to Geophysical Research Letters, 27(14), 2125-2128.

Oguz, T., Latif, M.A., Sur, H.I., Ozsoy, E., Unluata, U., 1991. On the dynamics of the southern Black Sea. In: E. İzdar, J.M. Murray (Eds.). Black Sea Oceanography, pp. 43-63. NATO-ASI Series C, 351, Kluwer Acad. Publ., Netherlands.

Oguz, T., Latun, V.S., Latif, M.A., Vladimir, V.V., Sur, H.I., Markov, A.A., Ozsoy, E., Kotovhchikov, B.B., Eremeev, V.V., Unluata, U., 1993. Circulation in the surface and intermediate layers of the Black Sea, Deep Sea Res. 40, 1597-1612

Oguz, T., Ducklow, H., Malanotte-Rizzoli, P., Tugrul, S., Nezlin, N.P., Unluata, U., 1996. Simulation of annual plankton productivity cycle in the Black Sea by a one-dimensional physical-biological model. J. of Geophys. Res. 101(C7) 16, 585-599.

Richardson, T.L., 1991. Comparison of ^{14}C primary production determinations made by different laboratories. Mar. Ecol. Prog. Ser. 72, 189-201.

Richardson T.L., Cullen, J.J., Kelley, D.E., Lewis, M.R., 1998. Potential contributions of vertically migrating Rhizosolenia to nutrient cycling and new production in the open- ocean. J. Plankton Res. 20 (2), 219-241.

Shaffer, G., 1986. Phosphate pumps and shuttles in the Black Sea. Nature 321, 515-517.

Shuskina, E.A., Musaeva, E.I., 1990. Structure of planktonic community from the Black Sea epipelagical and its changes as the result of the introduction of a ctenophore species. Oceanology 30, 306-310.

Steemann-Nielsen, E., 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J. Cons. Int. Explor. Mer. 18, 117-140.

Tugrul, S., Basturk, O., Saydam, C., Yilmaz, A., 1992. Changes in the hydrochemistry of the Black Sea inferred from water density profiles. Nature 359, 137-139.

Vedernikov, V.I., Demidov, A.B., 1993. Primary production and chlorophyll in the deep regions of the Black Sea. Oceanology 33, 229-235.

Vedernikov, V.I., Demidov, A.B., 1997. Vertical distribution of primary production and chlorophyll during different seasons in deep regions of the Black Sea. Oceanology 37 (3), 376-384.

Yilmaz A., Yunev, O.A., Vedernikov, V.I., Moncheva, S., Bologa, A.S., Cociasu, A., Ediger, D., 1998a. Unusual temporal variations in the spatial distribution of chlorophyll-a in the Black Sea during 1990-1996. In: L.I. Ivanov , T. Oğuz, (Eds.) Ecosystem Modeling as a Management Tool for the Black Sea, , Vol. 1, pp.105-120. Kluwer Academic Publishers, Netherlands.

Yilmaz, A., Tugrul, S., Polat, C., Ediger, D., Coban, Y., Morkoc, E., 1998b. On the production, elemental composition (C,N,P) and distribution of photosynthetic organic matter in the southern Black Sea. Hydrobiologia 363, 141-156