JOURNAL OF MARINE SYSTEMS

Long-term changes in nutrient supply of phytoplankton growth in the Black Sea

Alexander S. Mikaelyan [a,*], Andrey G. Zatsepin [a], Valeriy K. Chasovnikov [b]

[a] P.P.Shirshov Institute of Oceanology RAS, Nakhimovsky Prosp. 36, Moscow 117997, Russia
[b] Southern Branch of P.P.Shirshov Institute of Oceanology RAS, Prospornaya str., Gelendzhik 353470, Russia

ABSTRACT

During the period from 1970s to 2000s in the open waters of the Black Sea ecosystem has passed the stage of eutrophication, which manifested in high values of chlorophyll *a*, biomass of phytoplankton, primary production, etc. The goal of current research is to trace the long-term (1969–2008) dynamics in supply of nutrients and their ratios in the pycnocline, as well as changes in intensity of the upward flow of nutrients into the euphotic zone and impact of these factors on abundance and the taxonomic structure of phytoplankton. Based on changes of the total phytoplankton biomass three periods were revealed: Pre-eutrophication (PR) in 1969–1983, Eutrophication (EU) in 1984–1995 and Post-eutrophication (PS) in 1996–2008. The cold intermediate layer (CIL) in the Black Sea is formed by winter convection and store attributes of its intensity during the subsequent period of the year. Based on this, changes of nutrient content in this layer, as well as in the upper layer of the main pycnocline (Pycnocline) were used to track long-term variations of the annual bottom-up flux of nutrients to the euphotic zone. Data on nutrient content (P-PO_4^-, N as N-NO_3^- + N-NH_4^+ and Si) and their ratios were analyzed both for the CIL and the Pycnocline. Averaged depth-integrated phytoplankton biomass increased 6 times from 3.3 g m^{-2} in PR to 19.6 g m^{-2} during EU and decrease down to 10.6 g m^{-2} in PS, what was still 3 times higher than during PR. Similar changes were observed in N both in the CIL and in the Pycnocline. N in the CIL averaged 1.2, 3.3 and 2.5 μM in PR, EU and PS, respectively. P has increased significantly on 30% from 0.28 μM during PR to 0.37 μM in PS, while Si sharply decreased from 20 μM in PR to 12.1 μM in PS. Nutrient ratios changed similarly in the CIL and in the Pycnocline. In the CIL, N:P ratio increased from 6.4 in PR to 11.2 in EU and then dropped down to 8.8 in PS. Si:N ratio decreased from PR to PS from 19.6 to 7.3. Different algae groups predominated in the total phytoplankton biomass: dinoflagellates in PR (58%), diatoms in EU (47%) and both diatoms and coccolithophorids (31% and 28%) in PS. Comparative analysis of nutrient dynamics and changes in taxonomic structure of phytoplankton communities showed dependence of dinoflagellates and diatoms on high N and N:P ratios. Decreasing Si did not affect diatoms while probably it limited a growth of silicoflagellates. Increase of abundance of coccolithophorids during recent decades was governed by high P.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Obvious source of inorganic nutrients in euphotic zone in the open waters of the Black Sea is the bottom-up flux from the upper part of pycnocline layer, caused mainly by wind-induced vertical mixing during the winter convection processes (Oguz, 2008). Nutrients from the upper part of the main pycnocline down to depth of nitrate maximum located in density layer of ~15.4 sigma-t are washed away in the upper mixed layer providing the main annual nutrient flux to euphotic zone. After formation of seasonal thermocline in spring this winter mixed water is locked in the Cold Intermediate Layer (CIL), which formally is bounded by isotherm of 8 °C. Its parameters depend upon severity of previous winter conditions. For example, average water temperature in cold years is lower on 1.1 °C in comparison with that in warm years (Titov, 2003). The thickness of the CIL and water volume also is higher in abnormally cold years in 2.5–2.6 times. The CIL is formed both in the central part of the sea and on shelf areas first of all in the north-western part (Ivanov et al., 1997; Ovchinnikov and Popov, 1987). The contribution of these two sources is still debated. According to resent estimates the main volume of the total CIL water mass is formed in the central sea area, while shelf waters contribute from 16 to 25% (Polonsky and Popov, 2011). Part of this water penetrates directly in the CIL and the other is mixed with surface open waters due to horizontal advection. Both these processes load nutrients in the sea basin. Nevertheless, this contribution presumably is not the main source of 'new' nutrients in the deep sea area. For instance, only 20% of nitrates consumed by phytoplankton in the open waters are the allochthonous origin (McCarthy et al., 2007). Vertical flux of nutrients from the pycnocline is a key factor which

* Corresponding author. Tel.: +7 499 124 59 74; fax: +7 499 124 59 83.
E-mail addresses: mikaelyan@ocean.ru (A.S. Mikaelyan), zatsepin@ocean.ru (A.G. Zatsepin), chasovn@mail.ru (V.K. Chasovnikov).

supplies phytoplankton growth in sea interior and supports new production (Yunev et al., 2005).

During the warm period in April–November the CIL is located below the seasonal thermocline and euphotic zone on 40–100 m depending on sea regions (Titov, 2003, 2004b) which prevents consumption of nutrients with phytoplankton. Due to low vertical mixing between the CIL and layers above and below changes in this layer are not substantial during a warm season (Piotukh et al., 2011). Therefore, variations of nutrient content (high or low nutrient concentrations) in this layer as well as in the upper part of the main pycnocline could be used for tracing changes in the winter bottom-up flux.

During the last decade's, drastic changes in content of nutrients occurred in the coastal areas in the north-western part and western shelf of the Black Sea where the increase in inorganic nitrogen and phosphorus and decrease in dissolved silicate were observed (Cociasu and Popa, 2005; Oguz and Velikova, 2010; Tugrul et al., 1992). It was hypothesized that these changes were caused by a powerful anthropogenic eutrophication in 1970s and 1980s and with some delay appeared in the open waters during the period from mid-1980s to mid-1990s (Konovalov and Murray, 2001; Yunev et al., 2005). Gradual changes of maximum of nitrate were observed from ~2 μM in 1969 to ~4–5 μM in 1970s and to ~6–8 μM in the 1990s and then to ~5–6 μM in the second half of 1990s (Konovalov and Murray, 2001; Oguz and Gilbert, 2007; Tugrul et al., 1992; Yunev et al., 2005). In contrast to inorganic nitrogen weak (less than 20%) reduction of inorganic phosphorus was reported in the upper layer (above depth of 14.8 sigma-t) for the period between 1969 and 1990s (Konovalov and Murray, 2001). Obvious decrease in content of dissolved silicate was observed from the beginning of the 1970s to the 1990s both in surface waters and in the CIL (Humborg et al., 1997; Konovalov and Murray, 2001; Tugrul et al., 1992). Most of these long-term observations were completed in early 1990s. Since the mid-1990s, the dynamics of hydrochemical parameters in the open waters is poorly known. The last made observations indicated a possible change of some long-term trends.

Anthropogenous impact on a shelf did not significantly influence an ecosystem of the deep part of the Black Sea in the 1970s and to mid-1980s (Kideys, 2002). Sharp changes in functioning of pelagic ecosystem have been recorded in the mid-1980s (Oguz and Gilbert, 2007). On increase of phytoplankton biomass, chlorophyll *a* and primary production were reported in many studies (Mikaelyan, 1997; Nesterova et al., 2008; Yunev et al., 2002, 2005). This regime shift occurred under the influence of eutrophication in the open waters and coincided with the onset of regional cold climatic period since the mid-1980s, which ended in the mid-1990s (Oguz and Gilbert, 2007). The hypothesis according to which, gradual accumulation of a stock of nutrients at a depth temporarily did not affect surface layer was stated. From the beginning of the cold period with powerful winter convection this stock of nutrients became available to a phytoplankton in euphotic zone that led to sharp increase in primary production (Yunev et al., 2005). It could be assumed that changes of hydrochemical environment at a depth and the bottom-up flux of nutrients before the eutrophication period, in time and after had to determine not only the abundance of planktonic algae, but also the taxonomical structure and species composition of phytoplankton communities.

The goal of current research is to trace the long-term (40 years) dynamics in supply of nutrients and their ratios in the subsurface layers in the open waters of the Black Sea, changes of bottom-up fluxes of nutrients to euphotic zone at different stages of eutrophication and the effects of these processes on the biomass and taxonomic structure of the phytoplankton.

2. Materials and methods

Long-term changes in phytoplankton communities were analyzed using the Black Sea phytoplankton data base with the addition of more recent data (Mikaelyan et al., 2007). For the deep basin phytoplankton biomass under water column and taxonomic structure of the community were available from 1969 to 2008. Data on phytoplankton were analyzed for sea area with depths more than 100 m. The total number of stations with depth-integrated phytoplankton biomass was equal to 245 (Table 1).

Data on nutrient content (P-PO$_4^-$, N-NO$_3^-$, N-NH$_4^+$ and Si) were collected using different sources. Recent data were mainly obtained in regular cruises to the north-eastern and central part of the sea from 1999 till 2007 (P. P.Shirshov's Institute of Oceanology RAS, Moscow, Russia). Historical data from 1969 were taken from NATO TuBlack Project data base (Ivanov et al., 1998), SESAME European Project data base (http://isramar.ocean.org.il/sesamemeta) and NOAA (http://www.nodc.noaa.gov). Initially quality check of data was executed by expert groups of projects or scientists — data providers. After collecting base, additional procedures on quality control (doubling data, outlying values) were carried out. Eventually the total number of samples which are available for the deep basin (seabed depths more than 200 m) exceeded 29,000 (Table 2).

Stations were distributed over sea area more or less evenly (Fig. 1). In each sample the content of at least one nutrient was measured. Analytical methods for the estimations of nutrient concentrations partially differ in the riparian countries of the Black Sea and some distinction in values can be caused by distinctions in modification of used technology (Konovalov et al., 1994). In the current research we analyze average data for the long period and we believe that in this regard the general tendencies could not significantly be masked or distorted by these methodological peculiarities. Analysis of long-term trends was performed for concentrations of inorganic nitrogen (N, as sum of nitrates and ammonia), phosphorus (P), silicon (Si) and their ratios.

The ratios between nutrients are very sensitive to extreme values resulting in these cases to extremely high or low values which seriously influence average values. Because of these, core with 95% of data was used, i.e. 2.5% from the lowest and from the highest parts of range was excluded from the analysis.

Vertical distribution of physical properties in the Black Sea is different between central and peripheral areas. Permanent pycnocline is located at a depth of 60 to 100 m in the central part of the sea in the areas of two cyclonic gyres, and at a depth of 100 to 180 m or more on the outskirts of the sea (Titov, 2004a). In this regard the analysis of temporary changes was carried out for 4 ranges of isobaths: 200–1000 m, 1000–1500 m, 1500–1800 m and more than 1800 m.

For biological data standard statistical analysis ANOVA was used. For chemical parameters the preliminary analysis showed that some of them don't correspond to normal distribution. In this regard, for the nutrients statistical significance of difference between the means was assessed using the nonparametric Kruskal–Wallis test. Mean values in the text below are given with standard error ($\pm$SE) and the number of measurements (n).

3. Results

3.1. Phytoplankton biomass alterations

During 40 years total phytoplankton biomass in the warm period (mean for May–October) varied in the wide range from 1.5 to 41 g m^{-2}, while the average for all years was 14.2 g m^{-2} (Fig. 2a).

Table 1
Phytoplankton data base statistics.

Years	1969–2008
Data sets	45
Stations (>100 m)	245
Samples	1676
Species entries	58,066

Table 2
Nutrient data base statistics.

Year	Months	Datasets	Stations	Samples
1969	Mar, Apr	1	35	548
1970	Feb	1	5	113
1973	Mar, Apr	1	42	268
1974	Apr, Jul	1	4	23
1978	Sep, Oct	1	10	111
1980	Feb, Mar, Apr	1	29	217
1981	Sep, Oct	1	45	364
1984	Apr, May	1	46	671
1985	Oct	1	19	76
1986	Jan, May, Jun, Jul, Sep	5	43	342
1987	Apr, May, Aug	3	49	423
1988	Mar, Apr, May, Jun, Jul, Aug, Sep, Nov	5	232	3693
1989	Jan, Feb, Mar, Apr, Jul, Aug, Sep, Nov, Dec	7	185	2202
1990	Feb, Apr, Sep, Oct	4	134	1737
1991	Feb, Mar, Apr, Jun, Sep, Oct, Nov, Dec	6	221	3428
1992	Jul, Sep, Oct	4	202	3193
1993	Apr, Aug, Nov, Dec	7	229	2355
1994	Apr, May, Sep, Oct, Dec	6	198	2469
1995	Mar, Apr, Jul	4	106	1457
1996	Apr, Jun, Jul, Sep, Oct	3	96	1232
1997	Jul	1	49	440
1998	Jun, Sep	2	8	47
1999	Jun, Sep, Oct, Dec	8	93	1350
2000	Apr, Sep, Oct, Nov, Dec	7	69	735
2001	Apr, Jun, Sep, Nov	6	36	427
2002	May, Aug	2	2	394
2003	Apr, May	2	18	344
2004	May, Jun, Dec	3	5	142
2005	Jun	1	1	16
2006	May, Aug	3	6	144
2007	Jun, Aug, Sep[a]	4	6	157
	Total	102	2223	29118

Sea bottom >200 m.

High phytoplankton biomass was observed from mid-1980s to mid-1990s. This period coincided in time with severe winters caused deep winter convection and reflected in low sea surface temperature (Fig. 2a). Period of high phytoplankton biomass more clearly revealed in the interannual biomass anomalies — deviations from the average of 40 years (Fig. 2b). One of the simplest methods for the detection of different periods is the method of cumulative sum based on variations of mean (Rodionov, 2005). The minimum and maximum points on the curve of cumulative sum mark the beginning and end of the period with high abundance of phytoplankton (Fig. 2b), which corresponds well to the period of eutrophication in the open waters of the Black Sea (Oguz and Gilbert, 2007). On the basis of long-term changes in phytoplankton biomass 3 periods with different state of phytoplankton community were identified: Pre-eutrophication (PR) in 1969–1983, Eutrophication (EU) in 1984–1995 and Post-eutrophication (PS) in 1995–2008. Average of integrated phytoplankton biomass under water column for PR was 3.3 ± 3.8 g m^{-2} (n = 23) and increased by 6 times during EU to 19.6 ± 2.0 g m^{-2} (n = 88). During PS it decreased to 10.6 ± 2.4 g m^{-2} (n = 64), but nevertheless it was 3 times higher than during PR. Average for EU is statistically different (p < 0.05) from both PR and PS.

3.2. Nutrient vertical and spatial distribution

After the formation of the seasonal pycnocline in March and up to December the winter mixed water is retained in the CIL. Because of this, the interannual changes in nutrient content in this layer can be considered as an indicator for changes in bottom-up flux of nutrients. In order to avoid spatial differences in vertical distribution of physical and chemical properties vertical profiles were analyzed versus density. Usually, in a warm season CIL occurs in density range from 14 to
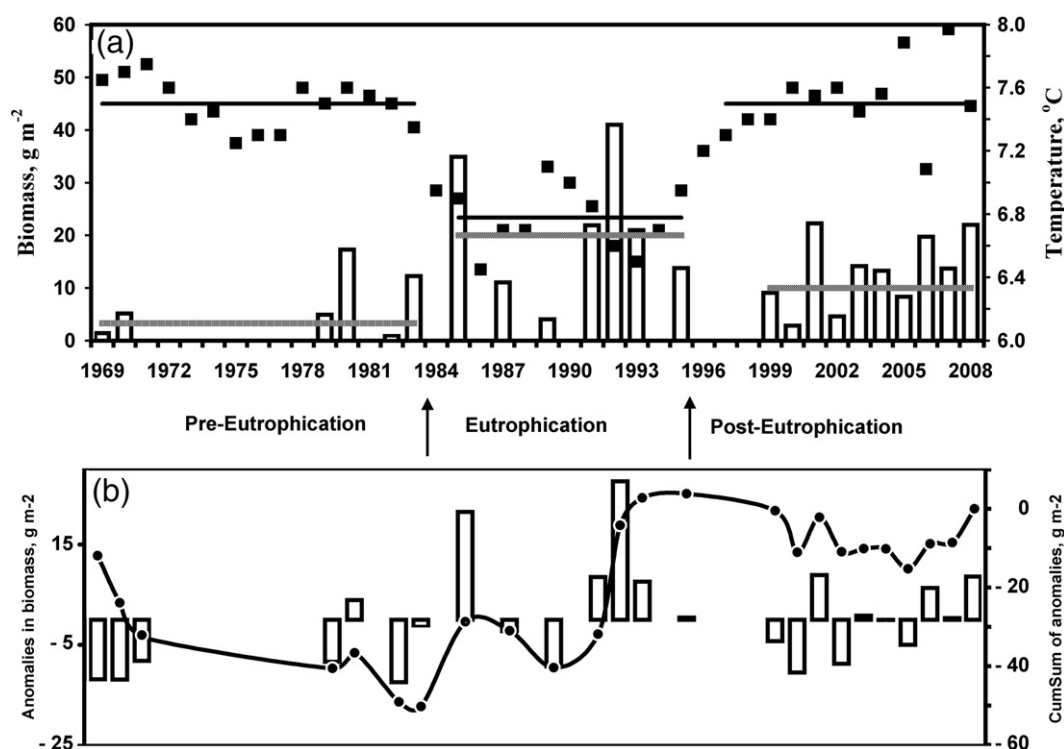

Fig. 1. Long-term changes of total phytoplankton biomass (g m^{-2}) in the open waters (see bottom depths >100 m) during May–October and mean winter December–March SST °C. a) Columns — mean annual total phytoplankton biomass; dots — winter SST (after Oguz et al. (2008) with addition of recent years); solid and dashed lines — mean SST and mean phytoplankton biomass for Pre-eutrophication, Eutrophication and Post-eutrophication periods. b) Columns — annual anomalies of mean in phytoplankton biomass; line — cumulative sum of the anomalies.
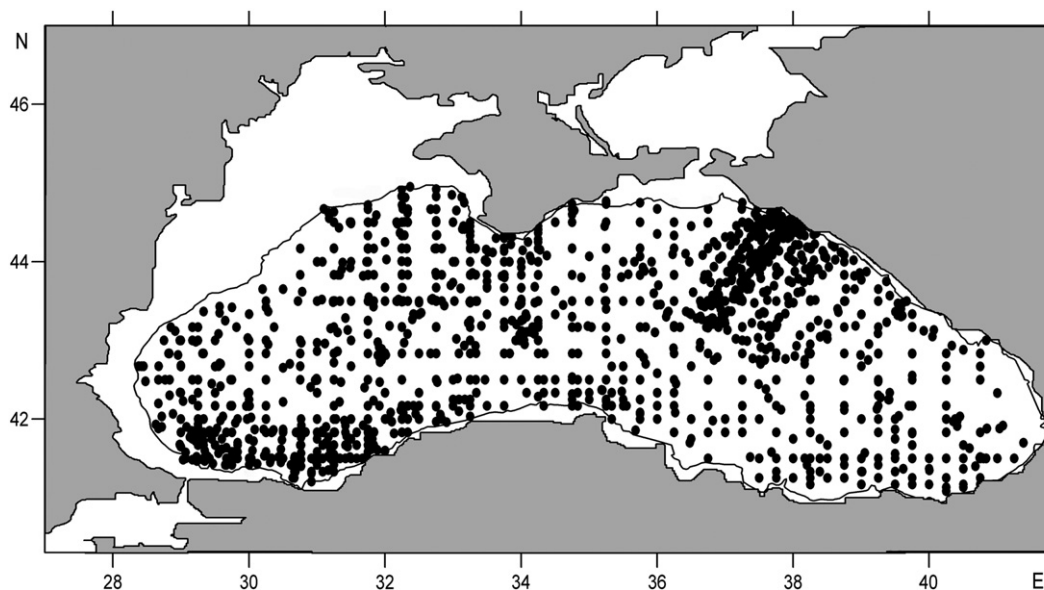
Fig. 2. Locations of stations in the open waters of the Black Sea (sea bottom depths > 200 m) used for chemical data analysis.

15.2 sigma-t (Fig. 3a). On an average for the period from May to October, the temperature profile shows that the core of this layer with low temperature gradients was located between 14.3 and 14.9 sigma-t. This layer was used in order to monitor changes in nutrient concentrations and below, for shortness, it will be denoted as a CIL.

Nutrients penetrate to euphotic zone from the upper part of the main pycnocline. Thus, concentration of nutrients in this layer can be used as an indicator of the potential for upward flow. Layer from 15.2 to 15.6 sigma-t was chosen for this analysis. In this layer the maximum of nitrates is located (close to 15.4 sigma-t) and ammonia is distributed more or less evenly (Fig. 3b). Simultaneously vertical profile of phosphates demonstrates a small preliminary peak very important from the point of view of their access to the upper layers,

while silicates decreased gradually (Fig. 3c). For shortness, the layer between 15.2 and 15.6 sigma-t will be denoted below as a Pycnocline.

N (represented as a sum of nitrate and ammonia) showed weak increase trend in Pycnocline from the peripheral to the central part of the sea (Fig. 4). During PR trend was evident, but this could be due to the low number of measurements (n = 9) for this period in the sea area adjacent to the shelf. The same trend was not found in PS and was very weakly expressed in EU. During EU throughout the sea N was higher than in the other two periods. Means for PR, EU and PS were 5.3 μM, 7.2 μM and 5.2 μM, respectively (Table 3). During PR and PT, increase in N from outskirts to the central sea area was observed also in the CIL (Fig. 4). Conversely, N clearly decreased in the same direction during EU. In this period, N was almost three times lower in the central
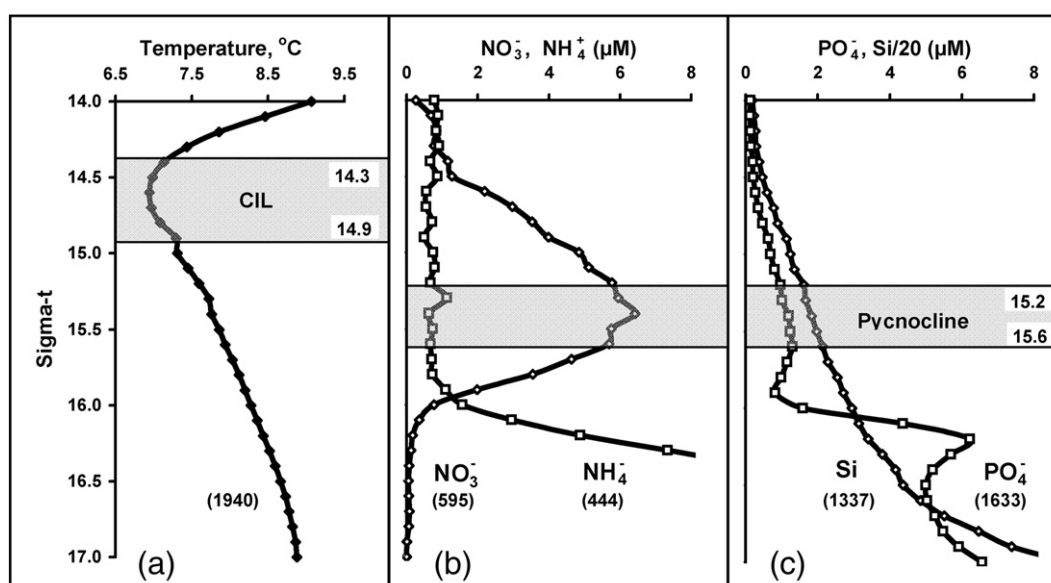

Fig. 3. Average May–October vertical distribution of temperature (a), nitrates and ammonia (b), silicates and phosphates (c) versus water density (sigma-t) in subsurface layer of the water column. Grey areas show position of core of the CIL (a) and layer in the upper part of Pycnocline (b, c) which were chosen for nutrient concentration analysis. Values in white rectangles show upper and lower boundaries of sigma-t. Numbers of averaged profiles are shown in brackets.
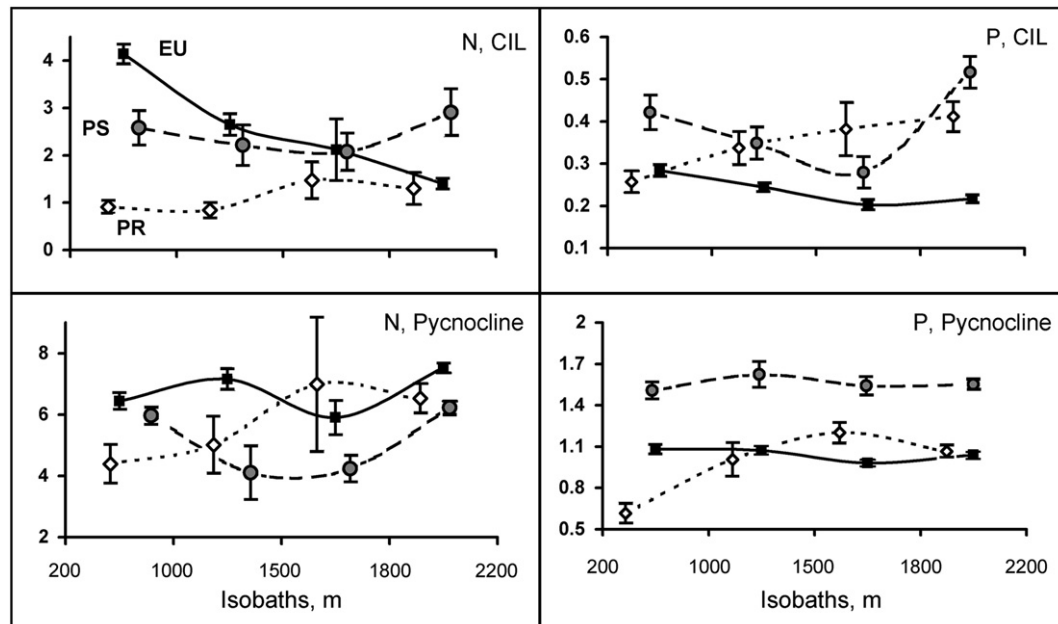
Fig. 4. Mean (April–November) concentration (µM) of inorganic nitrogen (N, $NO_3 + NH_4$), phosphorus (P) in the CIL (top panels) and in the Pycnocline (bottom panels) in 4 ranges of isobaths during Pre-eutrophication (PR), Eutrophication (EU) and Post-eutrophication (PS) periods. Vertical bars show SE.

region (1.4 ± 0.11 µM, n = 52) than in the near shelf (4.1 ± 0.2 µM, n = 230) regions (Fig. 4).

A similar pattern was observed in the distribution of P (Fig. 4). In the Pycnocline, during EU and PS trends were absent, while in PR, content of P increased from periphery toward the center of the sea in 2 times. The mean of P for all sea area was highest in PS (1.5 µM) in comparison with PR and EU (Table 3). In the CIL, like in the case with N the highest P was measured in the center of the sea during PR and PS (Fig. 4). On the contrary, during EU period P decreased in direction off shelf (0.28 ± 0.01 µM, n = 450) to the central sea area (0.21 ± 0.01 µM, n = 949).

3.3. Case study

Weak trends in nutrient content or their absence in the Pycnocline are expected given the relatively static hydrochemical properties of this layer. A slight increase in nutrient content in the central part of

Table 3
Mean concentrations (µM ± SE) of nutrients and their ratios during 3 periods.

Parameter	Pre-eutrophication 1969–1983	Eutrophication 1984–1995	Post-eutrophication 1996–2007
Pycnocline, all deep sea basin >200 m			
N	5.3 ± 0.5 (31)	7.2 ± 0.13 (171)	5.2 ± 0.16 (135)
P	1.0 ± 0.03 (152)	1.0 ± 0.01 (1399)	1.5 ± 0.02 (511)
Si	39.9 ± 0.99 (68)	37.8 ± 0.3 (1163)	36.2 ± 0.5 (504)
Pycnocline, central deep sea basin >1500 m			
N	5.7 ± 0.6 (24)	7.4 ± 0.16 (118)	5.3 ± 0.19 (104)
P	1.1 ± 0.03 (96)	1.0 ± 0.011 (853)	1.5 ± 0.026 (350)
Si	43.2 ± 0.78 (98)	37.6 ± 0.38 (672)	34.7 ± 0.61 (342)
N:P	4.9 ± 0.29 (21)	6.5 ± 0.12 (113)	3.9 ± 0.15 (75)
Si:N	9.7 ± 0.7 (24)	5.7 ± 0.31 (112)	6.2 ± 0.31 (71)
Si:P	40 ± 1.4 (87)	38 ± 0.46 (627)	25 ± 0.7 (301)
CIL, peripheral deep sea basin 200–1500 m			
N	1.2 ± 0.11 (39)	3.37 ± 0.17 (107)	2.45 ± 0.28 (22)
P	0.28 ± 0.019 (137)	0.27 ± 0.009 (860)	0.37 ± 0.024 (184)
Si	19.9 ± 0.6 (123)	10.7 ± 0.25 (684)	12.1 ± 0.47 (181)
N:P	6.4 ± 0.96 (36)	11.2 ± 0.6 (95)	8.8 ± 1.37 (18)
Si:N	19.6 ± 0.9 (37)	5.26 ± 1.34 (87)	7.3 ± 0.6 (87)
Si:P	97 ± 5.8 (107)[a]	69 ± 2.5 (579)	62 ± 4.6 (170)

Number of measurements is given in brackets.

the sea in the CIL is well consistent with the concept of an intense upward flow over the two central cyclonic gyres during winter convection, and with the fact that the thickness of this layer in the central part of the sea is minimal (Phiotukh et al., 2011). Reduced content of nutrients in the center of the sea during EU does not correspond to the described pattern and points to unusual situation where another factor determines the concentration of biogenic elements in the CIL. This factor can be shown on an example of transect across the sea carried out in February 1991. During this severe winter the main pycnocline lifted up at depth of 30–40 m below surface in the centers of both eastern and western cyclonic gyres. This induced the powerful bloom of phytoplankton in the center of the sea represented mainly by diatom species *Pseudo-nitzschia pseudodelicatissima* (Mikaelyan, 1995). This bloom lasted over a month and occurred on a repeated transect at the beginning of March. The total phytoplankton biomass reached up to 3 g m^{-3}, on some stations the integrated biomass under water column amounted up to 70–125 g m^{-2} (Mikaelyan, 1995). This phenomenon was observed earlier during the cold winter in 1988 when the high values of chlorophyll *a* and primary production were measured in the western central part of the sea (Krupatkina et al., 1991).

With the forming of seasonal pycnocline and termination of winter convection in mid March nutrients are rapidly consumed by high phytoplankton biomass. The bloom terminated and the water with depleted nutrients is locked in the CIL. That is why, in cold years with severe winters concentration of nutrients in the CIL in the center of the sea cannot be used as an indicator of bottom-up flux in wintertime. This gives an explanation of unusual spatial distribution of nutrients in the CIL during EU (Fig. 4). Based on these findings and considerations, long-term changes in nutrients in the CIL were analyzed for periphery of the sea from 200 m to 1500 m bottom depths, where they are more conservative and located well below euphotic zone. In turn, changes in potential of the bottom-up flux were traced in the Pycnocline in the central part of the sea with depths more than 1500 m.

3.4. Nutrient changes in the CIL and in the Pycnocline

During 40 years mean annual concentration of nitrates in the CIL varied from 0.3 to 8.2 µM. The highest values were observed in 1984–1993 (Fig. 6). The same pattern was observed in the Pycnocline.

N (nitrates + ammonia) generally followed by this dynamics was showing the highest values in 1984–1989 both in the CIL and in the Pycnocline. P demonstrated an increasing trend in both layers especially in the period from 1998. On the contrary, Si decreased gradually in the CIL and in the Pycnocline. These data series are based on not regular observations with different number of measurements per year. Due to this, only general trends could be detected and correct statistical comparison could be done using an averaging for the long-term periods.

N in the CIL was 3 times higher during EU than that in PR (Fig. 7a, Table 3). After EU content of N decreased on 35% to 2.45 μM. This value was not statistically different (p = 0.08) from that in EU but was significantly (p = 0.02) higher in 2 times than during PR. P significantly increased on ~40% from 0.27 μM during EU to 0.37 μM in PS. Si sharply decreased from 20 μM in PR to 10.7 μM during EU and stayed on approximately the same level in PS. As a whole, changes of the nutrients in the Pycnocline in the central sea area were similar to those in the CIL on the sea periphery (Fig. 7b, Table 3). N increased on 30% from 5.75 μM in PR to 7.4 μM during EU, and then decreased on 40% in PS. P increased on 50% from 1.0 μM during EU to 1.5 μM in PS. Si decreased from 43 μM during PR to 35 μM in PS.

Nutrient ratios changed in a similar way in the CIL and in the Pycnocline (Fig. 8, Table 3). In the CIL, N:P ratio increased from 6.4 in PR to 11.2 in EU and then dropped down to 8.8 in PS (Fig. 8a). In the Pycnocline, N:P ratio followed the same dynamics, but values were lower than in the CIL (Fig. 8b). Means for PR, EU and PS were equal to 4.9, 6.5 and 3.9, respectively. Si:P ratio decreased from 97 during PR to 62 during PS in the CIL. The same means for the Pycnocline were 40 and 25. Si:N ratio sharply decreased from PR to EU both in the CIL (from 19.6 to 5.2) and in the Pycnocline (from 9.7 to 5.7). In both layers this ratio slightly increased during PS.

Distribution of N:P ratios showed that only in several cases they were higher than Redfield stoichiometric average ratio equal to 16. This level was exceeded in PR, EU and PT only in 8%, 9% and 17% of samples, respectively. In the Pycnocline they were lower in all samples. During all periods in both layers Si:N ratio was higher than stoichiometric average ratio which is approximately equal to 1. Si:P ratio in the CIL was lower than stoichiometric average (~15) in 1%, 7% and 14% of all cases during PR, EU and PS, respectively. The similar trend increasing in time was observed in the Pycnocline. The same values were equal to 1%, 4% and 10%, respectively.

3.5. Taxonomic structure of phytoplankton

Different changes were observed in various taxonomic groups of phytoplankton (Fig. 9). Biomass of dinoflagellates during EU (7.9 ± 1.6 g m^{-2}) was significantly higher than that in PR (1.3 ± 3.2 g m^{-2}) and PS (2.3 ± 1.9 g m^{-2}). The role of this group in phytoplankton community decreased from PR to PT. Biomass of diatoms changed in similar pattern like dinoflagellates. Highest biomass was observed during EU (8.1 ± 1.0 g m^{-2}). It was significantly higher than in PR (1.8 ± 1.9 g m^{-2}) and PS (3.5 ± 1.2 g m^{-2}). It should be noticed that in PR mean biomass of diatoms was higher than that of dinoflagellates. However, the latter more often dominated the phytoplankton communities, which explains the higher average share of this group in the total phytoplankton biomass. For diatoms this value increased from 33% in PR to 47% in EU and decreased to 31% in PS (Fig. 10). The same values for dinoflagellates were equal to 58%, 36% and 22%, respectively.

Another pattern was observed in a case of coccolithophorids (Fig. 9). During PR their biomass was low (0.05 ± 0.08 g m^{-2}) and sharply increased during EU (1.9 ± 0.4 g m^{-2}) and further in PS (2.7 ± 0.5 g m^{-2}). Their share in the total phytoplankton biomass also increased drastically from 1.3% in PR to 28% in PS (Fig. 10). On the contrary, biomass of silicoflagellates demonstrated an opposite trend. Mean biomass decreased from PR (0.08 ± 0.03 g m^{-2}) to PS

(0.02 ± 0.02 g m^{-2}) in 4 times. During PR they have played a substantial role in the phytoplankton communities of the Black Sea. They comprised 8% of the total phytoplankton biomass. During EU and in the aftermath this value decreased to 0.3–0.5%.

During all three periods taxonomic structure of phytoplankton communities differed (Fig. 10). In PR, dinoflagellates prevailed in phytoplankton biomass at a noticeable role of diatoms and silicoflagellates. In EU, diatoms contributed a highest portion to the total phytoplankton biomass with significant share of dinoflagellates and coccolithophorids. In PS, the proportion of diatom, and coccolithophorids in the total biomass of phytoplankton were approximately equal. In addition, the role of "other algae" represented mainly by small non-identified flagellates increased. Biomass of this group and its contribution to the total phytoplankton biomass has increased several-fold. However, we do not trust the trend, because in the former times, small flagellates usually were underestimated due to low quality of the equipment and inability to identify them on species level.

4. Discussion

Eutrophication in the deep waters of the Black Sea at the end 1980s and in early 1990s was documented in many studies as increase of the water-column-integrated phytoplankton biomass (Mikaelyan, 1997; Nesterova et al., 2008), chlorophyll concentration and primary production (Vedernikov and Demidov, 2002; Yunev et al., 2002) and as decrease in water transparency according to Secchi disc measurements (Mankovsky et al., 1998). For the period from the mid-1980s to the mid-1990s, which is referred to as the intensive phase of eutrophication, the exact terms have not been clearly defined. The latter is crucial for understanding the factors responsible for this phenomenon. Some authors believe that reduction of top-down control of zooplankton on phytoplankton biomass has played important role during EU (Oguz and Gilbert, 2007). Long-term changes in zooplankton community were marked by rather high level of biomass in the 1980s, a sharp decline in 1990 due to income of invader ctenophore Mnemiopsis leidyi and the subsequent recovery of the zooplankton abundance in the late 1990s (Kovalev et al., 1998; Vinogradov, 1990). However, these substantial from 2 to 15 g m^{-2} changes in zooplankton standing stock were not correlated with corresponding increase and decrease in phytoplankton abundance. Based on this mismatch it could be postulated that grazing press was not an important regulator of proliferation of phytoplankton during EU. On the contrary, ideal (year by year) coincidence of the beginning of the cold climatic period on regional scale in 1984 and its ending in 1995 with the period of high phytoplankton biomass (Fig. 1) shows that this regime shift at low trophic level to high algae abundance and back was a result of changes in bottom-up flux of nutrients to the euphotic layer. Accordingly, regime shift at higher trophic level from high to low zooplankton biomass and back was out of touch with the cold period and was apparently caused by other factors, including those mentioned above.

In current study, content of nutrients in the CIL was used as an indicator of bottom-up flux to the euphotic zone. In warm months, this water mass remains almost unchanged because of the low vertical mixing. From April to November, the average temperature does not change significantly in the CIL slightly increasing by 0.3–0.4 °C (Ivanov and Belokopytov, 2011). Seasonal dynamics in the vertical distribution of nitrate is not expressed (Konovalov and Murray, 2001; Yunev et al., 2002). Such conservatism of physical and chemical properties in the CIL gives the grounds for annual averaging for warm months. At the same time, the horizontal exchange in the CIL occurs throughout the year. After winter, CIL water formed in the sea center extends to the periphery within 3–4 months and mixes up with the waters formed during 1–2 previous years (Ovchinnikov and Popov, 1987). This makes it difficult to analyze interannual fluctuations, but averaging at a time scale of several years gives the reliable values. A special case is the winter phytoplankton bloom in the central part

of the sea during severe winters (Fig. 5). In this case, the content of nutrients in the CIL is not a proper indicator of the upward flow to the euphotic zone. That is why, exception of the central part of the sea from the analysis, although it reduces the amount of data, allows attaining a more real picture of long-term changes of nutrients in the CIL. We believe that the observed increase of N in the CIL on the periphery of the sea during EU reflects the eutrophication process (Fig. 4). In addition, these changes in the CIL were consistent with the alterations in the Pycnocline in the center of the sea (Figs. 7 and 8), which also confirm the correctness of our understanding of the CIL, as the layer in which changes in the bottom-up flux of nutrients is "recorded".

Changes in concentration of nitrates in the Pycnocline have been reported earlier. On average, the maximum concentration of nitrate increased from ~2 μM in 1969 to ~4–5 μM in 1970s and to ~6–8 μM in the 1990s and then decreased to ~5–6 μM in the second half of the 1990s (Konovalov and Murray, 2001; Oguz and Gilbert, 2007; Tugrul et al., 1992; Yunev et al., 2005). As a whole, similar pattern was observed in the upper part of the pycnocline in our study (Fig. 6). N has increased from 5.7 μM in PR to 7.4 μM in EU and then has decreased to 5.3 μM in PS (Table 3). Long-term dynamics of nitrates in the CIL have not been reported earlier, but similar changes were documented for depth of summer chlorophyll maximum which is located above this layer. Concentration of nitrates has increased from ~0.1 μM in the 1970s to ~0.2 μM in the 1990s and then decreased back to ~0.1 μM in the mid-1990s (Yunev et al., 2005). Dynamics in the CIL revealed that N has declined after EU, but its concentration was significantly (p = 0.03) higher than before EU (Fig. 7). This could be considered as an evidence of more intensive bottom-up flux and more comfortable environment for growth of phytoplankton in PS compared to conditions which occurred in PR.

In contrary to N, concentration of P did not change remarkable from PR to EU in the CIL and slightly decreased on 7% (p = 0.002) in the Pycnocline (Fig. 7). These results are not in contradiction with weak (less than 20%) depletion in P content which was observed in the upper layer above depth of 14.8 sigma-t from 1969 to 1990s (Konovalov and Murray, 2001). The most conspicuous feature in dynamics of P was a sharp increase on 30–50% in PS both in the CIL and in the Pycnocline. Increase in phosphate concentrations in 2 times was also observed in the upper 15-m layer in summer-autumn period after 1990s (Mikaelyan et al., 2011). These observations indicate a higher availability of P to phytoplankton growth in the PS even in comparison with the EU.

The obvious tendency to decrease Si was observed both in the CIL and in the Pycnocline (Fig. 7). The fall of Si in the interior of the basin was reported earlier. In surface waters Si decreased by an order of magnitude from 1969 to 1988 (Humborg et al., 1997; Tugrul et al., 1992). Sharp decline from ~15 μM to 4 μM was observed in the layer above the CIL at depths of 14.0 sigma-t in oxic zone from early 1970s to 1990s (Konovalov and Murray, 2001). In the CIL, according to our findings Si fell down in 2 times from PR (~20 μM) to EU (~11 μM) and stayed on this level during PS (Table 3). In the Pycnocline, further decline was observed also after EU. This trend means a decrease in the bottom-up flux of Si into euphotic zone that can probably limit growth of some siliceous phytoplankton species.

Ratios between different nutrient species varied in a similar way both in the CIL and in the Pycnocline while absolute values differed (Fig. 8, Table 3). In the CIL, N:P ratios during PR, EU and PS were 1.3, 1.7 and 2.3 times higher compared with those in the Pycnocline. This discrepancy could be caused by additional nitrogen which was loaded in the CIL from shelf areas. During severe winters, besides the central part of the sea, the CIL also is formed in shelf regions mainly in the north-western shelf. According to calculations under
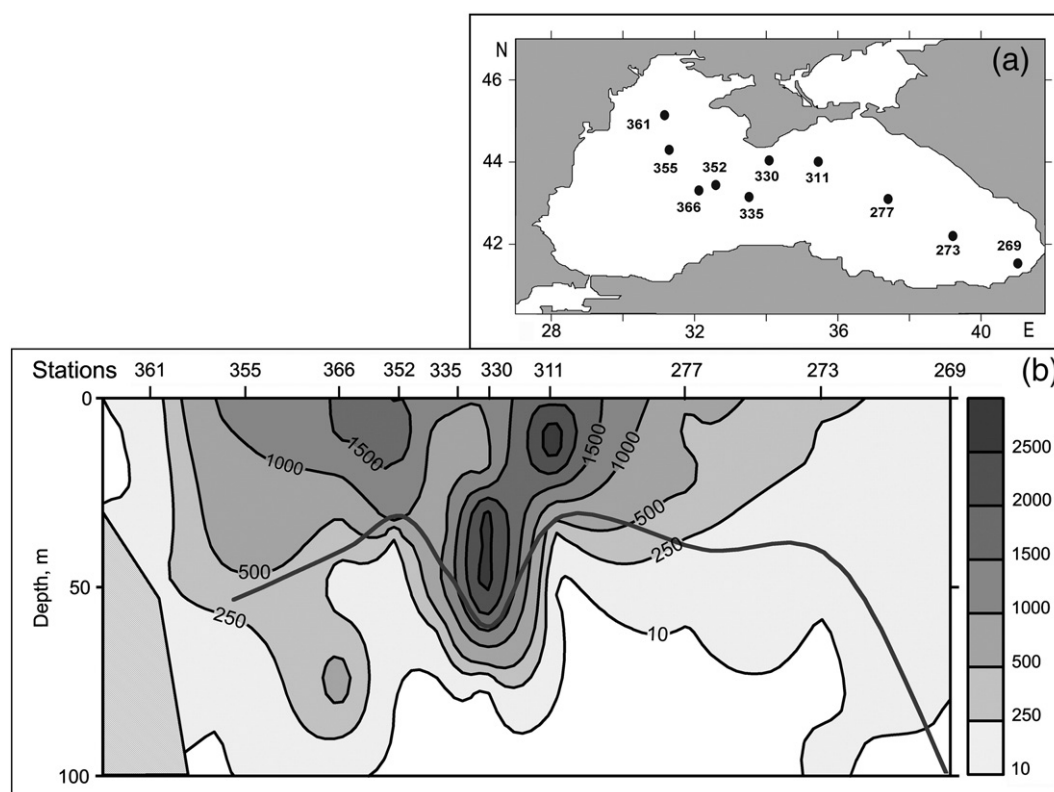

Fig. 5. Vertical distribution of the total phytoplankton biomass (μg l^{-1}) along transect across the Black Sea in February 1991. (a) Station locations; (b) the total phytoplankton biomass versus depth (m). Solid black line shows position of the main pycnocline.
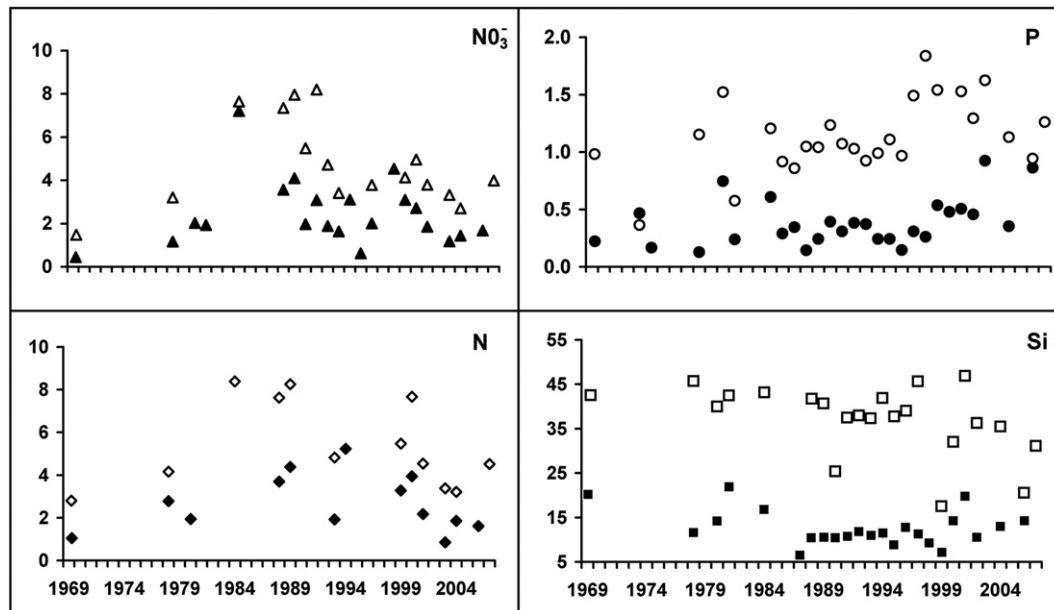
Fig. 6. Mean April–November concentration (μM) of nitrates (NO₃), inorganic nitrogen (N, NO₃ + NH₄), phosphorus (P) and silicates (Si) in the CIL (white symbols) and in the Pycnocline (black symbols) from 1969 to 2007.

severe winter conditions up to 50% of the CIL waters can be formed in these areas (Ivanov et al., 1997), while during warm winters this process does not occur at all (Polonsky and Popov, 2011). These nitrogen-rich shelf waters penetrate directly in the CIL or, at first, extend with numerous mesoscale eddies or intrusions of surface waters into the open water (Zatsepin et al., 2003) and finally deliver inorganic nitrogen in the CIL via organic particle sinking and complex remineralization processes (Karl and Knauer, 1991; Konovalov and Murray, 2001). The observed increase over time of difference in N:P ratio between the CIL and the Pycnocline is consistent with rise in nitrate concentrations in the north-western shelf from ~5 μM in 1970s to ~15 μM in 2000s (Cociasu and Popa, 2005; Oguz and Velikova, 2010).

In both the CIL and the Pycnocline during all three periods N:P ratio was lower than canonical Redfield ratio 16:1. This ratio is not

an optimum for growth of any species, but represents mainly an average of species-specific values. In dependence on temperature, light conditions and species it varies in broad range from 4 to 160 (see review of Zhang and Hu, (2011)). Optimum N:P ratio estimated by model under different environmental conditions gave values ranging from 8 to 45 (Klausmeier et al., 2004). In general, it is considered that on a long-term time scale assimilation of nitrogen and phosphorus by phytoplankton should be approximate to a ratio of 16:1. One can assume that N:P ratio less than 10 indicates nitrogen limitation, and the same ratio above 20 indicates phosphorus limitation of phytoplankton growth in many species (Glibert and Buckholder, 2011). From this point of view permanently low N:P ratio from 4 to 7 in the Pycnocline (Table 3.) means strong nitrogen limitation "programmed" in bottom-up flux into the euphotic zone. Similar
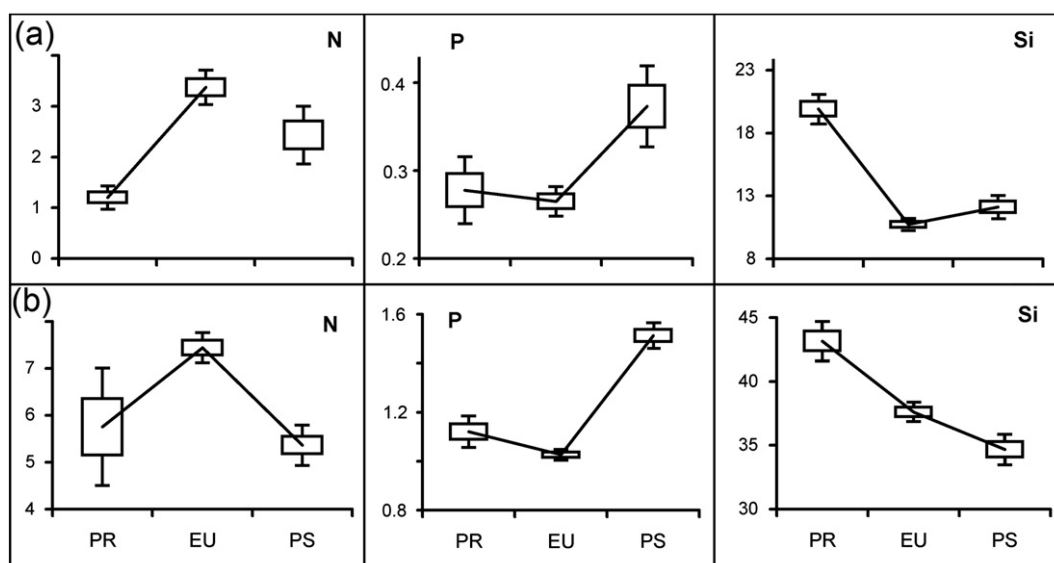

Fig. 7. Mean April–November concentration (μM) of inorganic nitrogen (N, NO₃ + NH₄), phosphorus (P) and silicates (Si) in the CIL (a) and in the Pycnocline (b) during Pre-eutrophication (PR), Eutrophication (EU) and Post-eutrophication (PS) periods. Line links values with significant difference of means (Kruskal–Wallis test, p < 0.05); rectangles show − SE; vertical bars − 2SE.
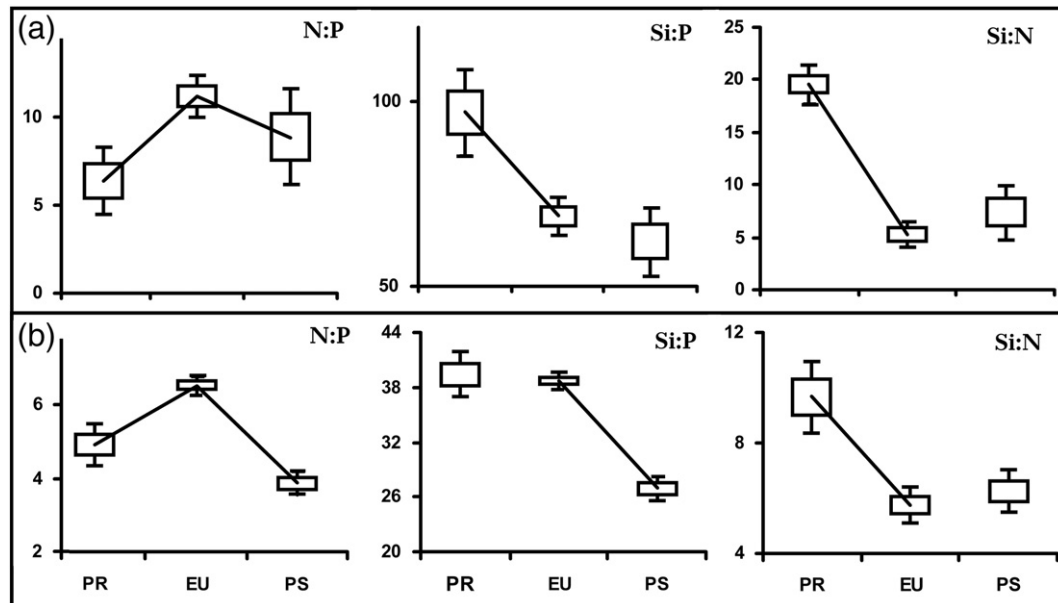
Fig. 8. Mean April–November nutrient ratios (concentration μM) of the main nutrients N:P, Si:P and Si:N in the CIL (a) and in the Pycnocline (b) during Pre-eutrophication (RR), Eutrophication (EU) and Post-eutrophication (PS) periods. Line links values with significant difference of means (Kruskal–Wallis test, p < 0.05); rectangles show − SE; vertical bars − 2SE.

low N:P ratios from 2.5 to 6.5 were observed in the upper nutricline in both central gyres and in the outlying areas in the early 1990s (Basturk et al., 1997). This nitrogen limitation decreased significantly in the EU, but then returned to the level of PS (Fig. 8). Addition of terrestrial nitrogen increases the stock of N in the upper layer and mitigates the nitrogen limitation, as evidenced by higher N:P in the CIL compared with the Pycnocline. This was especially substantial during EU when N:P ratio in the CIL was higher than 10. It can be assumed that during this period, nitrogen and phosphorus were more or less balanced, which provided favorable conditions for phytoplankton growth.

These findings are in line with representations on N-limitation of phytoplankton growth in the central basin of the Black Sea (Yilmaz et al., 2006). Experiments which were conducted with natural phytoplankton communities in the open waters also revealed the N-limitation in most cases (Krivenko, 2008; Yayla et al., 2001). The temporal changes of the total phytoplankton biomass as well as biomass of dinoflagellates and diatoms (Fig. 9) corresponded well to changes in N (Fig. 7) and N:P ratio (Fig. 8) in the CIL. This coincidence is consistent with the general ideas that the development of the two groups of algae in the open water depends mainly on the availability of nitrogen in the environment.
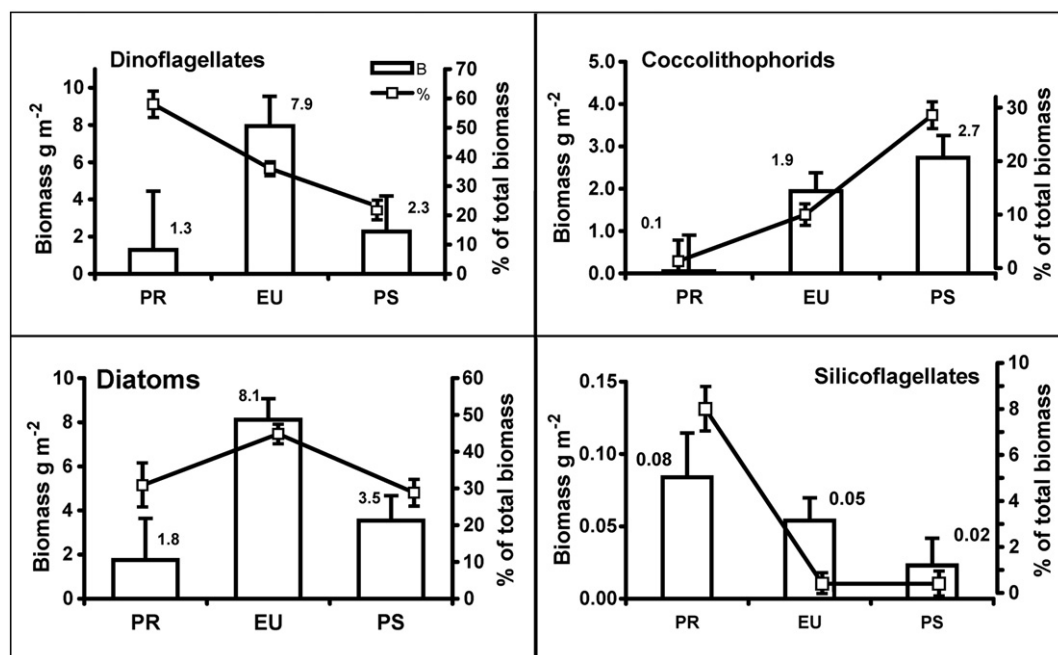

Fig. 9. Mean May–October biomass (g m^{-2}) of different taxonomic groups of phytoplankton under the water column (rectangles) and their share (%) in the total phytoplankton biomass (line) during Pre-eutrophication (RR), Eutrophication (EU) and Post-eutrophication (PS) periods. Values of figure show biomass values. Vertical bars − SE.

Remarkable changes of Si were not coherent with diatoms dynamics. It decreased gradually in the Pycnocline and fell down twice in the CIL from PR to PS (Fig. 7). Nevertheless, in PS, Si averaged ~4 μM in the upper mixed layer. This value is higher than concentrations limiting the growth of diatoms (1 to 3 μM) which are observed for most diatom species in experiments (Martin-Jézéquel et al., 2000). In nature, for example, Si > 2 μM was sufficient to maintain constant predominance by diatoms in Norwegian waters (Egge and Aksnes, 1992). Experiments performed in the open waters of the Black Sea also showed that silicate was not a limiting nutrient of phytoplankton growth in natural populations (Yilmaz et al., 2006). It can be assumed that Si did not limit growth of diatoms in the interior of the basin during all observed time period.

Unlike diatoms and dinoflagellates, coccolithophorids number and their share in the phytoplankton biomass gradually increased (Figs. 9 and 10). This trend in general coincided with increase in P content in the Pycnocline and in the CIL (Fig. 7). The same positive relationship between changes in biomass of coccolithophorids and P was revealed for the last 40 years in the upper 10 m layer in the Black Sea (Mikaelyan et al., 2011). Until 1992 average May–June phosphate concentration was equal to 0.21 μM, and for the period after 1991 it increased up to 0.4 μM, while the biomass of coccolithophorids for the same periods averaged to 45 μg l^{-1} and 175 μg l^{-1}, respectively. Series of experiments performed during several years both on shelf and in the open waters also confirmed P-limitation of coccolithophorids growth in May–June (Silkin et al., 2009). During these months regular annual bloom of

coccolithophorids occurs in the Black Sea. May–June observations from 2004 to 2009 showed that high abundance of coccolithophorids was observed simultaneously with high P in the upper 15 m layer (Mikaelyan et al., 2011). Share of this group of algae in the total phytoplankton biomass was 56–69% in years when P changed from 0.5 to 0.8 μM and 20–30% when P varied from 0.05 to 0.3 μM. Physiological properties of coccolithophorids also point out high demand in P in contrary to N. In several experiments, the limiting concentrations (considered as a half-saturation constant in nutrient uptake kinetics) were very low both for nitrate and ammonium and varied from 0.1 to 0.2 μM (Eppley et al., 1969), while for phosphates they were much higher and ranged from 0.3 to 0.5 μM in dependence of growth rate (Riegman et al., 2000). Other experiments showed a phosphate stress when concentrations fell below 0.25 μM (Dyhrman and Palenik, 2003). All of these relationships and the experimental results support the conclusion that in the Black Sea growth of coccolithophorids was primarily determined by the available P.

Long-term decrease of abundance of silicoflagellates (Fig. 9) coincided with a similar trend of Si both in the Pycnocline and in the CIL (Figs. 6 and 7). These algae have an internal silicic skeleton and like diatoms they acquire Si from the environment. To our knowledge, data on growth-limiting concentrations of N, P and Si are not known for this group. There are some data that at concentrations less than 1 μM of silicate growth rate of silicoflagellate species *Synura petersenii* greatly decreased (Klaveness and Guillard, 1975). Also it is known that cell morphology of silicoflagellates is sensitive to Si and at low
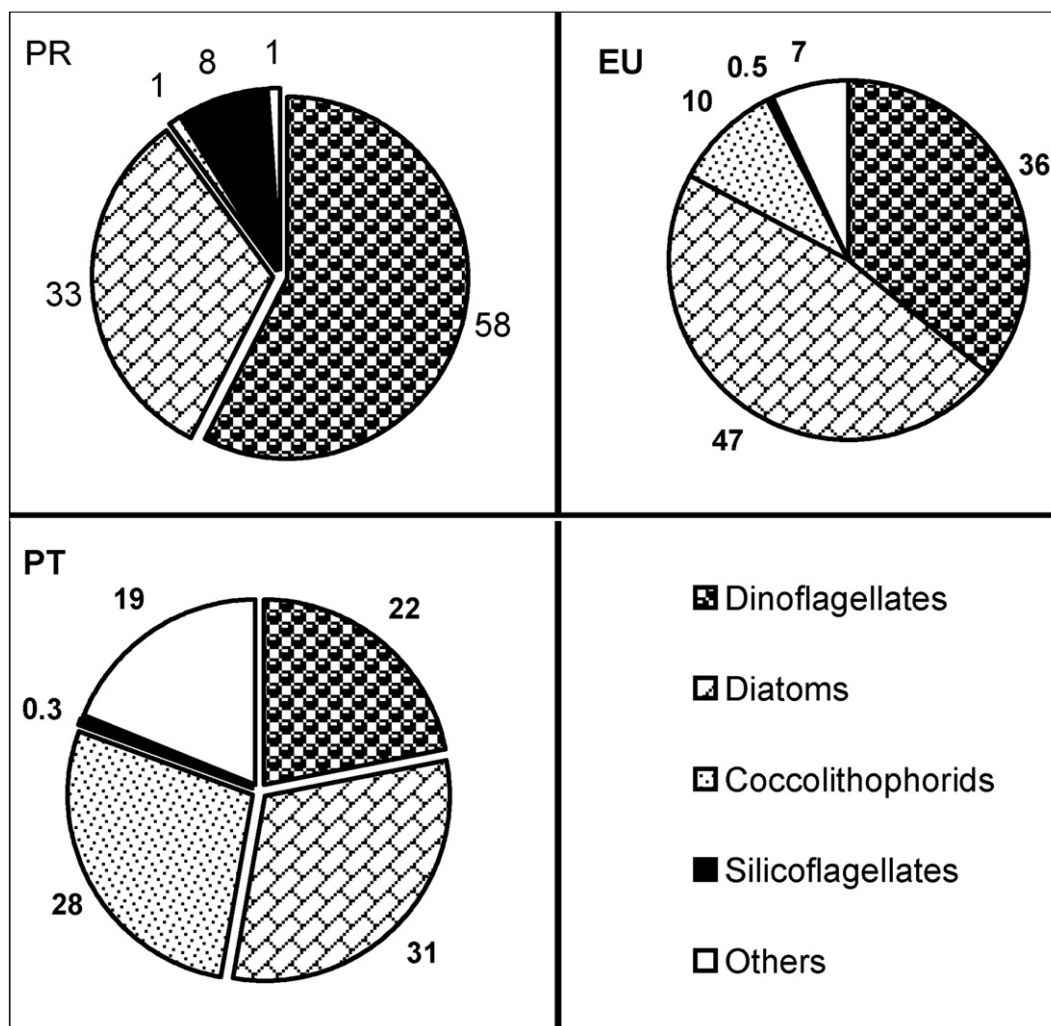

Fig. 10. Taxonomic structure of phytoplankton communities during Pre-eutrophication (RR), Eutrophication (EU) and Post-eutrophication (PS) periods. Mean May–October share (%) of 5 groups of algae in the total phytoplankton biomass under water column.

concentrations their skeleton is getting thinner up to total disappearance (Sandgren et al., 1996). Silicoflagellates are mainly developed in fertile areas of the oceans with the moderate-to-high level of nutrients (McCartney, 1993), that point out possible high growth-limiting concentrations of Si. With the lack of direct data on Si uptake kinetics it can be only assumed that unlike diatoms fall of Si in the upper layer could restrict development of silicoflagellates in the Black Sea.

As follows from many studies, the ratio of nutrients to a large extent determines the taxonomic composition of phytoplankton communities (Glibert and Buckholder, 2011; Philippart et al., 2000; Zhang and Hu, 2011). In many cases, high N:P ratio leads to the predominance of diatoms while the lower ratio often is favorable for dinoflagellates (Heil et al., 2007; Hodgkiss and Ho, 1997). In general, our results are in line with this opinion. Intensive bottom-up flux of N during harsh winters in EU caused the bloom of diatoms *Pseudo-nitzschia* sp. (Fig. 5), which prefers high nitrogen content (Carter et al., 2005). This increased amount of nutrients retained in the euphotic zone during summer. As a result, in the warm months during EU with balanced N:P ratio (>10 in the CIL) biomass of diatoms and dinoflagellates reached maximal values (Fig. 9). Meantime, diatoms more frequently prevailed in phytoplankton, and this has changed the community structure from predominance in the total biomass of dinoflagellates (58% in PR) to diatoms (47% in EU). Meanwhile, changes in N:P ratios were not reflected in dynamics of coccolithophorids. The same result was observed in the upper mixed layer in the Black Sea (Mikaelyan et al., 2011). It seems that growth of this group of algae mainly depends upon the absolute phosphate concentration. Some authors consider that decrease of Si:N ratio in the Black Sea has affected the species composition of phytoplankton and has led to the development of coccolithophorids and non-siliceous flagellates (Humborg et al., 1997). Despite the decrease in Si:N ratio current values (4–5 in the CIL) are much higher than 1, which is the balanced ratio for the growth of the most species of diatoms (Cloern, 2001; Egge and Aksnes, 1992; Turner et al., 1998). Besides, the dynamics of diatoms was not consistent with changes of Si:N ratio (Figs. 8 and 9). Based on these findings it could be suggested that the changes of Si:N ratio in the open water did not affect development of diatoms, but might have impact on reducing the abundance of silicoflagellates.

On the basis of a past and present condition of a hydrochemical background in the Black Sea the qualitative medium-term forecast on changes in community of a phytoplankton can be made. Climate regime in the region is characterized by cycles of warm and cold periods with approximate intervals of 15–25 years (Kazmin and Zatsepin, 2007; Oguz and Gilbert, 2007; Ovchinnikov and Popov, 1987). As from end of the previous cold period already passed 18 years, the probability of the next cooling period is quite high. Nowadays the potential of bottom-up flux of inorganic nitrogen is lower than that in EU (Fig. 7). The current N:P ratios are also lower (Fig. 8). On this basis, it can be assumed that within the next expected cold period the total phytoplankton biomass as well as biomass of diatoms and dinoflagellates will not reach the level of the EU. Due to high P further increase of share of coccolithophorids in the total phytoplankton biomass can be expected, while the low Si will define a minor role of silicoflagellates.

5. Conclusions

Long-term dynamics of phytoplankton showed an evident in 6 time increase in the total phytoplankton biomass during EU. After this period, biomass has decreased 2 times but was still 3 times higher than before eutrophication stage. Start of EU in 1984 and its termination in 1995 exactly coincided with cold climatic period on regional scale. This points out that "regime shifts" on lower trophic level to high phytoplankton biomass and back were result of changes in the bottom-up flux of nutrients into the euphotic layer in the open waters of the Black Sea.

N increased and decreased in a similar way both in the Pycnocline and in the CIL. In the CIL, during EU concentration of inorganic nitrogen was 3 times higher than in PR. After EU, N decreased but it was 2 times higher than before eutrophication period. This could be considered as an evidence of more intensive bottom-up flux of N and therefore more comfortable conditions for phytoplankton growth in PS compared with PR.

Other nutrients showed multidirectional dynamics. P considerably increased for 30–50% while Si gradually decreased 2 times in the CIL and by 25% in the Pycnocline. N:P ratio varied from 6.4 to 11.2 in the CIL and from 3.9 to 6.5 in the Pycnocline. Permanently low N:P ratio in the Pycnocline means strong nitrogen limitation coming with the bottom-up flux to the euphotic zone. This relative nitrogen deficiency was somewhat reduced in the EU, but afterward the situation had returned to a state of PS.

Changes of the total phytoplankton biomass as well as diatoms and dinoflagellates were similar to the dynamics of N. Their biomasses were highest during EU and decreased after, but remained at a higher level than before the eutrophication. Like P coccolithophorids showed a marked increase over time, which is consistent with their dependence on phosphate concentration. Abundance of silicoflagellates fell down in 4 times what coincided with decreasing trend in Si.

All these factors have changed the taxonomic structure of the phytoplankton communities from the predominance of dinoflagellates in the total biomass (58% in PR) and then to diatoms (47% in EU) and further to coccolithophorids together with diatoms (28 and 31% in PS). Based on the current nutrient concentrations and their ratios in the Pycnocline it can be assumed that within the next expected cold period the total phytoplankton biomass as well as biomass of diatoms and dinoflagellates will increase but will not reach the level of EU. Due to high P further increase of share of coccolithophorids in the total phytoplankton biomass can be expected, while the low Si probably will limit a growth of silicoflagellates.

References

Basturk, O., Turgul, S., Konovalov, S., Salihoglu, I., 1997. Variation in the vertical structure of water chemistry within the three hydrodynamically different regions of the Black Sea. In: Ozsoy, E., Mikaelyan, A. (Eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea. NATO-ASI Series, Environment, 27. Kluwer Academic Publishers, Dordrecht, pp. 183–196.

Carter, C.M., Ross, A.H., Schiel, D.R., Howard-Williams, C., Hayden, B., 2005. In situ microcosm experiments on the influence of nitrate and light on phytoplankton community composition. J. Exp. Mar. Biol. Ecol. 326, 1–13.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210, 223–253.

Cociasu, A., Popa, L., 2005. Significant changes in Danube nutrient loads and their impact on the Romanian Black Sea coastal waters. Cercet. Mar. 35, 25–37.

Dyhrman, S.T., Palenik, B., 2003. Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophorid *Emiliania huxleyi*. J. Plankton Res. 25, 1215–1225. http://dx.doi.org/10.1093/plankt/fbg086.

Egge, J.K., Aksnes, D.L., 1992. Silicate as regulating nutrient in phytoplankton competition. Mar. Ecol. Prog. Ser. 83, 281–289.

Eppley, R.W., Rogers, J.N., McCarthy, J.J., 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14, 912–920.

Glibert, M., Buckholder, J.M., 2011. Harmful algal blooms and eutrophication: "strategies" for nutrient uptake and growth outside the Redfield comfort zone. Chin. J. Oceanol. Limnol. 29, 724–738. http://dx.doi.org/10.1007/s00343-011-0502-z.

Heil, C.A., Revilla, M., Glibert, P.M., Murasko, S., 2007. Nutrient quality drives phytoplankton community composition on the West Florida shelf. Limnol. Oceanogr. 52, 1067–1078.

Hodgkiss, I.J., Ho, K.C., 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 852, 141–147.

Humborg, C., Ittekkot, V., Cociasu, A., v.Bodungen, B., 1997. Effect of Danube River dam on Black Sea biogeochemistry. Nature 386, 385–388.

Ivanov, V.A., Belokopytov, V.N., 2011. Oceanography of the Black Sea. NAS of Ukraine, Marine Hydrophysical Institute, Sevastopol (In Russian).

Ivanov, L.I., Besiktepe, S., Özsoy, E., 1997. The Black Sea cold intermediate layer. In: Ozsoy, E., Mikaelyan, A. (Eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea. NATO-ASI Series, Environment, 27. Kluwer Academic Publishers, Dordrecht, pp. 253–264.

Ivanov, L.I., Konovalov, S., Melnikov, V., Mikaelyan, A., et al., 1998. Physical, chemical and biological data sets of the TU Black Sea data base: description and evaluation. In: Ivanov, L., Oguz, T. (Eds.), NATO TU-Black Sea Project: ecosystem modeling as a management tool for the Black Sea. NATO-ASI Series, Environment, 47. Kluwer Academic Publishers, Dordrecht, pp. 11–38.

Karl, D.M., Knauer, G.A., 1991. Microbial production and particle flux in the upper 350 m of the Black Sea. Deep-Sea Res. 38 (Suppl. 2), S921–S942.

Kazmin, A.S., Zatsepin, A.G., 2007. Long-term variability of surface temperature in the Black Sea and its connection with the large-scale atmospheric forcing. J. Mar. Syst. 68, 293–301. http://dx.doi.org/10.1016/j.jmarsys.2007.01.002.

Kideys, A.E., 2002. Fall and rise of the Black Sea ecosystem. Science 297 (5586), 1482–1484. http://dx.doi.org/10.1126/science.1073002.

Klausmeier, C.A., Litchman, E., Daufresne, T., Levin, S.A., 2004. Optimal nitrogen-to-prosphorus stoichiometric of phytoplankton. Nature 429 (6988), 171–174.

Klaveness, D., Guillard, R.R.L., 1975. The requirement for silicon in Sinura petersenii. J. Phycol. 11, 349–355.

Konovalov, S.K., Murray, J.W., 2001. Variations in the chemistry of the Black Sea on a time scale of decades (1960–1995). J. Mar. Syst. 31, 217–243.

Konovalov, S., Romanov, A., Salihoglu, I., Basturk, O., Tugrul, S., Gokmen, S., 1994. Intercalibration of CoMSBlack-93a chemical data; unification of methods for dissolved oxygen sulfide analyses and sampling strategies CoMSBlack-94a cruise. Report of Institute of Marine Sciences, Erdemli.

Kovalev, A., Niermann, U., Melnikov, V., Belokopytov, V., Uysal, Z., Kideys, A.E., Unsal, M., Altukhov, D., 1998. Long-term changes in the Black Sea zooplankton: the role of natural and anthropogenic factors. In: Ivanov, L, Oguz, T. (Eds.), NATO TU-Black Sea Project: ecosystem modeling as a management tool for the Black Sea. NATO-ASI Series, Environment, 47. Kluwer Academic Publishers, Dordrecht, pp. 221–235.

Krivenko, O.V., 2008. Contents and uptake of inorganic nitrogen in the Black Sea. Morski Ecol. J. Ukraine 7, 13–26 (in Russian).

Krupatkina, D.K., Finenko, Z.Z., Shalapyonok, A.A., 1991. Primary production and size-fractionated structure of the Black Sea phytoplankton in the winter-spring period. Mar. Ecol. Prog. Ser. 73, 25–31.

Mankovsky, V.I., Vladimirov, V.L., Solovev, M.V., Besiktepe, S., 1998. Optical properties of the Black Sea: results of the CoMSBlack and TU-Black Sea Programs. In: Ivanov, L., Oguz, T. (Eds.), NATO TU-Black Sea Project: ecosystem modeling as a management tool for the Black Sea. NATO-ASI Series, Environment, 47. Kluwer Academic Publishers, Dordrecht, pp. 145–162.

Martin-Jézéquel, V., Hildebrand, M., Brzezinski, M.A., 2000. Silicon metabolism in diatoms: implications for growth. J. Phycol. 36, 821–840.

McCarthy, J.J., Yilmaz, A., Coban-Yildiz, Y., Nevins, J.L., 2007. Nitrogen cycling in the offshore waters of the Black Sea. Estuarine Coastal Shelf Sci. 74, 493–514.

McCartney, K., 1993. Silicoflagellates. In: Lipps, G.H. (Ed.), Fossil Prokaryotes and Protists. BlackWell, Cambridge, pp. 143–154.

Mikaelyan, A., 1995. Winter bloom of diatom Nitzschia delicatula in the open waters of the Black Sea. Mar. Ecol. Prog. Ser. 129, 241–251.

Mikaelyan, A.S., 1997. Long-term variability of phytoplankton communities in the open Black Sea in relation to environmental changes. In: Ozsoy, E., Mikaelyan, A. (Eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea. NATO-ASI Series, Environment, 27. Kluwer Academic Publishers, Dordrecht, pp. 105–116.

Mikaelyan, A.S., Pautova, L.A., Georgieva, L.V., Dyakonov, V.Yu., 2007. Data base on phytoplankton of the Black Sea. Oceanology 47, 447–480.

Mikaelyan, A.S., Silkin, V.A., Pautova, L.A., 2011. Coccolithophorids in the Black Sea: their interannual and long-term changes. Oceanology 51, 39–48.

Nesterova, D., Moncheva, S., Miakelyan, A.S., et al., 2008. The state of phytoplankton. In: Oguz, T. (Ed.), State of the Environment of the Black Sea (2001–2006/7). BSS, Istanbul, Turkey, pp. 173–192.

Oguz, T., 2008. General oceanographic properties: physico-chemical and climatic features. In: Oguz, T. (Ed.), State of the Environment of the Black Sea (2001–2006/7). BSS, Istanbul, Turkey, pp. 39–60.

Oguz, T., Gilbert, D., 2007. Abrupt transitions of the top-down controlled Black Sea pelagic ecosystem during 1960–2000: evidence for regime shifts under strong fishery exploitation and nutrient enrichment modulated by climate-induced variations. Deep-Sea Res. I 54, 220–242.

Oguz, T., Velikova, V., 2010. Abrupt transition of the northwestern Black Sea shelf ecosystem from a eutrophic to an alternative pristine state. Mar. Ecol. Prog. Ser. 405, 231–242.

Ovchinnikov, I.M., Popov, Yu.I., 1987. Formation of a cold intermediate layer in the Black Sea. Oceanology 27, 739–746.

Philippart, C.J.M., Cadeé, G.C., van Raaphorst, W., Riegman, R., 2000. Long-term phytoplankton–nutrient interactions in a shallow coastal sea: algal community structure, nutrient budgets, and denitrification potential. Limnol. Oceanogr. 45 (1), 131–144.

Phiotukh, V.B., Zatsepin, A.G., Kazmin, A.S., Yakubenko, V.G., 2011. Impact of winter cooling on the variability of thermohaline characteristics of active layer in the Black Sea. Oceanology 51, 221–230.

Polonsky, A.B., Popov, Yu.I., 2011. Conditions for Formation of the Cold Intermediate Layer in the Black Sea. MGI, Sevastopol (In Russian).

Riegman, R., Stolte, W., Noordeloos, A.A.M., Slezak, D., 2000. Nutrient uptake and alkaline phosphatase (EC 3:1:3:1) activity of Emiliania huxleyi (Prymnesiophyceae) during growth under N and P limitation in continuous cultures. J. Phycol. 36 (1), 87–96.

Rodionov, S.N., 2005. A brief overview of the regime shift detection methods. In: Velikova, V., Chipev, N. (Eds.), Large-scale disturbances (regime shifts) and recovery in aquatic ecosystems: challenges for management toward sustainability: UNESCO-ROSTE/BAS Workshop on Regime Shifts, 14–16 June 2005. IO BAS, Varna, pp. 17–24.

Sandgren, C.D., Hall, S.A., Barlow, S.B., 1996. Siliceous scale production in chrysophyte and synurophyte algae. I. Effects of silica-limited growth on cell silica content, scale morphology, and the construction of the scale layer of Synura petersenii. J. Phycol. 32 (4), 675–692.

Silkin, V.A., Pautova, L.A., Mikaelyan, A.S., 2009. Role of phosphorus in regulation of Emiliania huxleyi (Lohm.) Hay et Mohl. (Haptophyta) blooms in the northeastern Black Sea. Int. J. Algae 11 (3), 211–221.

Titov, V.B., 2003. On the correlation between seasonal atmospheric conditions and parameters of the hydrological water structure in the northeastern Black Sea. Oceanology 43, 325–332.

Titov, V.B., 2004a. Formation of the upper convective layer and the cold intermediate layer in the Black Sea in relation to the winter severity. Oceanology 44, 327–330.

Titov, V.B., 2004b. Integral effect of thermal and dynamical atmospheric factors on the hydrological structure of the Black Sea waters. Oceanology 44, 783–788.

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.

Turner, R.E., Qureshi, N., Rabalais, N., Dortch, Q., Justic, D., Shaw, R.F., Cope, J., 1998. Fluctuating silicate: nitrate ratios and coastal plankton food webs. Proc. Natl. Acad. Sci. U. S. A. 95, 13048–13051. http://dx.doi.org/10.1073/pnas.95.22.13048.

Vedernikov, V.I., Demidov, A.B., 2002. Long-term and seasonal variability of chlorophyll and primary production in the eastern regions of the Black Sea. In: Zatsepin, A.G., Flint, M.V. (Eds.), Multidisciplinary Investigations of the Northeast Part of the Black Sea. Nauka, Moscow, pp. 212–234 (In Russian).

Vinogradov, M.E., 1990. Investigation of the pelagic ecosystem of the Black Sea (44th cruise of the R/V Dimitriy Mendeleyev, July 4–September 17, 1989). Oceanology 30 (2), 254–256.

Yayla, M., Yilmaz, A., Morkoc, E., 2001. The dynamics of nutrient enrichment and primary production related to recent changes in the ecosystem of the Black Sea. Aquat. Ecosyst. Health Manage. 4 (1), 31–49.

Yilmaz, A., Coban-Yikdiz, Y., Telli-Karakoc, F., Bologa, A., 2006. Surface and mid-water sources of organic carbon by photoautotrophic and chemoautotrophic production in the Black Sea. Deep-Sea Res. II 53, 1988–2004.

Yunev, O.A., Vedernikov, V.I., Basturk, O., Yilmaz, A., Kideys, A.E., Moncheva, S., Konovalov, S., 2002. Long-term variations of surface chlorophyll-a and primary production in the open Black Sea. Mar. Ecol. Prog. Ser. 230, 11–28.

Yunev, O.A., Moncheva, S., Carstensen, J., 2005. Long-term variability of vertical chlorophyll a and nitrate profiles in the open Black Sea: eutrophication and climate change. Mar. Ecol. Prog. Ser. 294, 95–107.

Zatsepin, A.G., Ginzburg, A.I., Kostianoy, A.G., Kremenetskiy, V.V., Krivosheya, V.G., Poulain, P.M., Stanichny, S.V., 2003. Observation of Black Sea mesoscale eddies and associated horizontal mixing. J. Geophys. Res. 108 (C8), 3246. http://dx.doi.org/10.1029/2002JC001390 (1–27).

Zhang, Q., Hu, G., 2011. Effect on nitrogen to phosphorus ratios on cell proliferation in marine micro algae. Chin. J. Oceanol. Limnol. 29 (4), 739–745.