VARIABILITY OF INORGANIC NITROGEN FORMS IN ROMANIAN BLACK SEA MARINE AND COASTAL WATERS

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ABSTRACT

Seawater from a NW Black Sea transect was sampled in 9 stations with bottom depths within 15 – 2200 m during R/V "*Mare Nigrum*" cruise on 7-14 April 2008. The samples were frozen and subsequently analyzed in NIMRD "Grigore Antipa" laboratory for salinity, dissolved oxygen and nutrients (inorganic forms of phosphorus and nitrogen).

The purpose of the paper is to investigate the inorganic nitrogen forms and concentrations and control factors of water column distribution in NW Black Sea along a South-South East transect of Sf. Gheorghe area. The N* parameter test (Gruber and Sarmiento, 1997) was proposed to estimate nitrogen fixation-denitrification (marine nitrogen cycle significant stages) in NW Black Sea waters.

KEY WORDS: Black Sea, nutrient, nitrogen cycle, denitrification

INTRODUCTION

Nitrogen is one of life's bricks. It is essential in protein and nucleic acids synthesis. With a significant influence on major biogeochemical cycles (for example C or P) [1], nitrogen is the chemical element in sea water both in the molecular state (oxidation number, ON = 0) and compounds that may hold oxidation numbers from -3 to 5, making the marine nitrogen to have a complex chemistry. This variability includes both organic and inorganic nitrogen forms. Only some forms of nitrogen are biologically used but their availability has the key to ocean productivity [2].

In this respect, ammonia, nitrites and nitrates are the most studied mineral forms of nitrogen due to their importance in the primary productivity of marine waters. Each of these forms should be interpreted as a more or less advanced stage of organic matter degradation through bacterial action. Excepting the coastal areas frequently contaminated by land intake, the mineral salts dissolved in sea water can be interpreted as nitrogen cycle steps (Peres, 1963). Thus, whereas all forms of nitrogen appearance are highly influenced by oxygen environmental conditions, the study of the Black Sea waters, the largest permanently anoxic basin in the world (Murray, 1991), can provide important information for understanding the marine nitrogen cycle, being an attractive natural laboratory for marine chemists and microbiologists (Codispoti, l991).

The paper aim is to investigate the dominant forms of inorganic nitrogen concentrations in the NW Black Sea as well as the control factors of their distribution in the water column along a SSE transect of Sf. Gheorghe area. At the same time a model is proposed for nitrogen estimating nitrogen fixation and denitrification (important stages in marine nitrogen cycle) in coastal and deep waters of the NW Black Sea.

MATERIAL AND METHODS

Seawater samples were collected with a CTD SeaBird equipped with rosette and 5 liters Niskin bottles in the framework of international project SESAME, R/V "Mare Nigrum" oceanographic expedition, during 7-14 April 2008.

There were collected and analyzed 81 samples from the water column (0-500m) on a profile located S-SE of Sf.Gheorghe Danubian branch, consisting of 9 stations with bottom depths between 15 and 2200 m (Fig. 1). The samples were frozen and subsequently analyzed in the NIMRD “Grigore Antipa” measurements and analysis laboratory, where determinations were made for salinity, dissolved oxygen, nutrients (phosphorus and inorganic nitrogen forms).

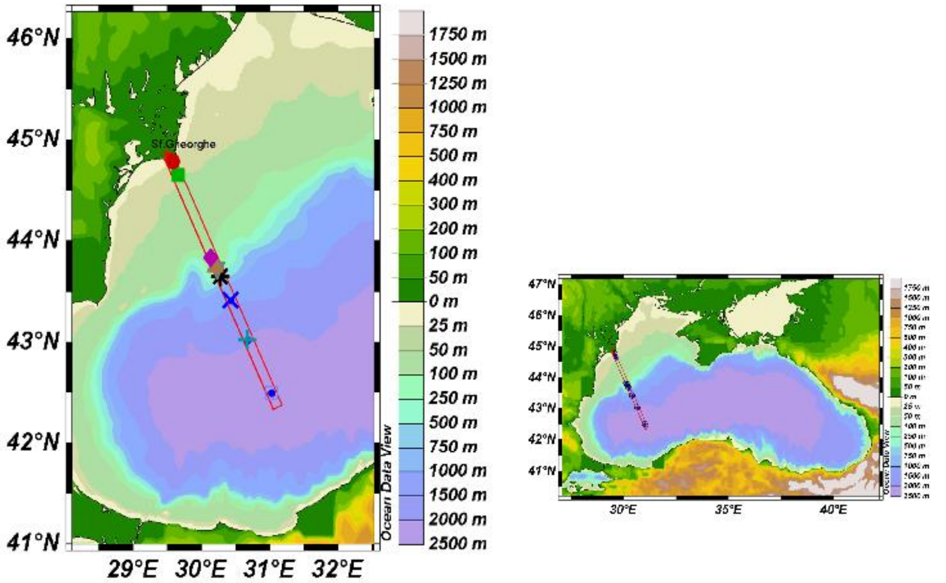


Fig. 1 - Map of stations performed during R/V "*Mare Nigrum*" expedition, April 2008

Seawater nutrients were quantified by spectrophotometric analytical methods validated in the accredited laboratory according to EN ISO 17025:2005 and having as reference manual "Methods of Seawater Analysis" (Grasshoff, 1999) with the following detection limits and extended relative uncertainties, coverage factor, 95.45% (Table 1).

Table 1

	Parameter	**UM**	**Limit of detection (µmol/dm^3)**	**Extended relative uncertainty, U(c), k=2, coverage factor 95.45%**
1.	$(NO_3)^-$	µM	0.12	c x 0.08 µmol/dm^3
2.	$(NO_2)^-$	µM	0.03	c x 0.06 µmol/dm^3
3.	$(NH_4)^+$	µM	0.12	c x 0.10 µmol/dm^3
4.	$(PO_4)^{3-}$	µM	0.01	c x 0.12 µmol/dm^3

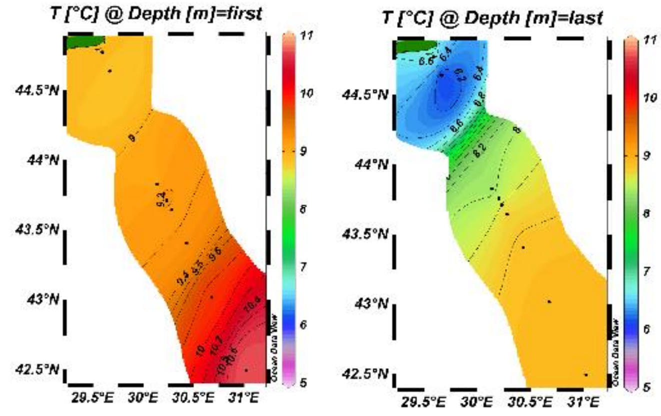
A Shimadzu UV-VIS spectrophotometer was used measuring within the range 0-1000 nm. Salinity was determined by using Mohr Knudsen method while dissolved oxygen by using Winkler method on board immediately after sampling.

The data were processed with Ocean Data View software version 4.0 (Schlitzer, 2009) and Microsoft Office Excel.

Results and discussions

1. General environmental indicators distribution

Horizontal distribution of temperature (Fig. 1.1) highlights both surface (a) and bottom (b) the increased gradient with distance from shore.



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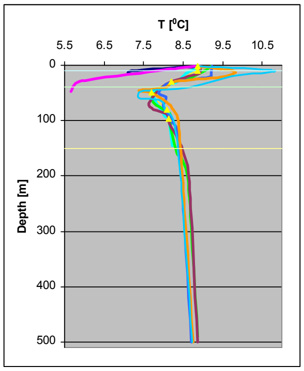
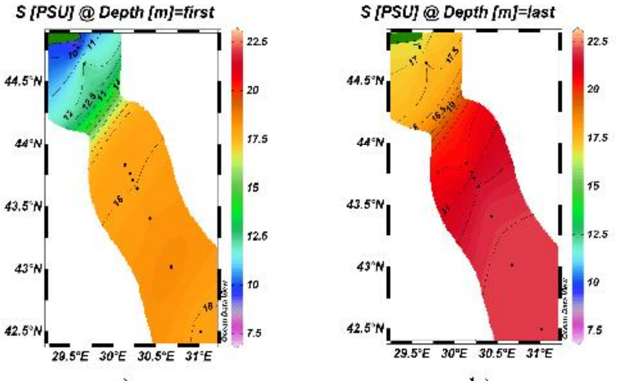


Fig. 1.1 - Seawater temperature horizontal distribution surface (a) and bottom (b) – "*Mare Nigrum*", April 2008

Fig. 1.2 - Seawater column temperature distribution – "*Mare Nigrum*", April 2008

Being under the direct influence of air temperature, seawater temperature (Fig.1.2) was lower in the shallow stations by minimum 5.67°C recorded in Station 2, at maximum depth of 47m. Maximum value - 10.81°C, was found in Station 9, at 10.1m depth. Although the temperature varies within a narrow range, the measurements showed the whole pattern of the Black Sea thermal stratification (Vespremeanu, 2004): the presence of mixed layer (0 – 10m depth), the seasonal weakly pronounced thermocline (10 - 40m depth), the Cold Intermediate Layer – CIL (40 – 150m depth) and the intermediate layer (150 – 500m depth). At 500m, the temperatures in Stations 5 - 9 ranged between 8.71 - 8.88^{o}C.

Concerning the salinity regime the minimum value (7.45 PSU) was recorded in Station 1 - surface layer, due to the Danube direct influence. Except for this value, salinity fitted within specific area and season range, 14.09 - 22.08 PSU. The maximum value is found in Station 9, at 500m depth. Thus, the horizontal distribution analysis (Fig.1.3) highlights the ascending gradient from inshore to offshore and also from surface to the bottom.



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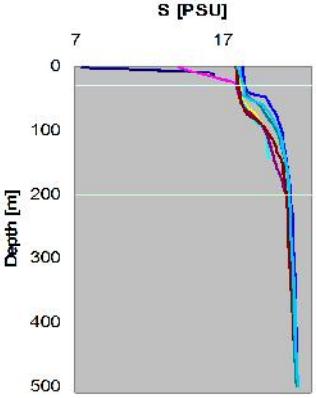

Fig. 1.3 - Seawater salinity horizontal distribution surface (a) and bottom (b) – "*Mare Nigrum*", April 2008

Fig.1.4-Seawater column salinity distribution – "*Mare Nigrum*", April 2008

Vertical distribution of salinity (Fig.1.4) clarifies the mixed layer (0 – 40m depth) where salinity ranges from 7.45-18.49 PSU, the halocline layer (40 – 200m depth) with values between and 18.20-21.62 PSU and the intermediate layer (200-500m) where there are slight variations of salinity such as 22.03 - 22.08 PSU.

Both temperature and salinity values, recorded in the 200 – 500m layer depth, are consistent with the fact that T = 8.5°C - 9°C and S = 22.32 PSU also characterize the deepest area of the Black Sea, being stationary for centuries (Konovalov and Murray, 2001).

Dissolved oxygen regime was maintained within the specific values of seasonal natural variability of the studied area. Being under the influence of temperature, water mass circulation and biological phenomena amplitude, dissolved oxygen values recorded homogeneous values at surface (0-20m) for the entire study area ranging from 7.01 - 7.38 cm^3/l, (313.1 - 329.6 μM) (Fig. 1.5a).

Bottom horizontal distribution of dissolved oxygen (Fig. 1.5.b) shows the parameter decreasing up to "undetectable" at depths greater than 150m. The oxycline layer is situated at 50 – 90m depth layer (into CIL) where concentrations decreased from about 7 cm^3/l to $1cm^3/l$. The suboxic layer, where concentrations fall below $1cm^3/l$ to almost zero is found in Stations 4, 5 and 6 between 100 – 150m and higher in Stations 7, 8 and 9, at 70 -100m depth (Fig.1.6).

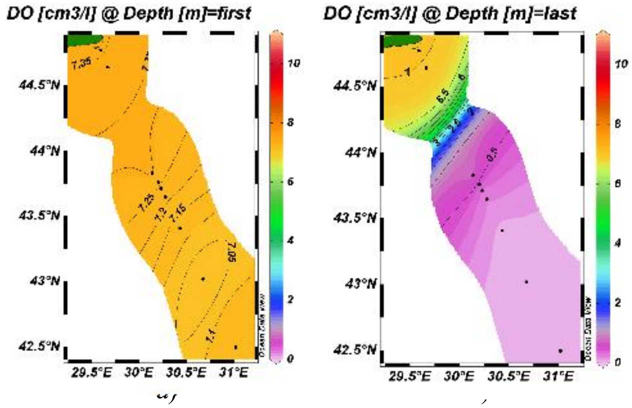

Fig. 1.5 - Seawater dissolved oxygen horizontal distribution surface (a) and bottom (b) – "*Mare Nigrum*", April 2008

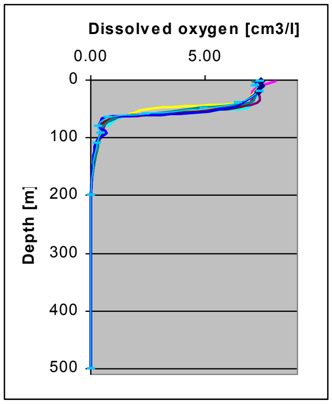

Fig. 1.6 – Seawater dissolved oxygen column distribution – "*Mare Nigrum*", April 2008

At depths greater than 200 m, dissolved oxygen is "undetectable", waters being anoxic.

2. Nitrogen electronic diversity – nitrogen compounds variability

Holding five relatively stable oxidation states in the marine environment, nitrogen, N, is very different from most other nutrients. With the same number of valence electrons, phosphorus, P, is found almost exclusively in the marine environment in the form of orthophosphate (PO_4^{3-}) with ON= +5. At the same time, nitrogen can be found in the marine environment as nitrate, NO_3^-(ON=+5), nitrite, NO_2^-(ON=+3), nitrous oxide, N_2O (ON=+1), molecular nitrogen, N_2 (ON=0) and ammonium NH_4^+ (ON=-3). In addition there are many organic compounds containing nitrogen, most often in the form of amino groups, $-NH_2$ (ON =-3). The reason why nitrogen has a large number of stable oxidation states is reflected in its electronic configuration, $1s^2 2s^2 2p^3$, a structure allowing releasing up to 5 e^-(ON=+5) or acceptance up to 3 e^-(ON=-3) to achieve a rare gas stable configuration. The required ionization energy for donating five valence electrons is relatively small so it can be compensated by the covalent bonds formation, which explains the relative stability of nitrate forms, NO_3^- and ammonium NH_4^+ but does not explain the stability of nitrite species, NO_2^-, molecular nitrogen, N_2, or nitrous oxide, N_2O[1] .

Seawater nitrogen cycle involves a series of complex transformations: (1) nitrates and nitrites reduction to nitrous oxide, molecular nitrogen, ammonia and organic nitrogen through assimilation or denitrification processes, (2) ammonium production by organic nitrogen decomposition (ammonification), (3) ammonium oxidation to nitrites, nitrates or nitrous oxide (nitrification) and (4) molecular nitrogen reduction to ammonium and organic nitrogen (N_2 fixation). Thus, the nitrogen cycle involves transfer of electrons between the oxidized (NO_3^-) and reduced (NH_4^+) forms. The phytoplankton uptake removes efficiently nitrates, nitrites and ammonium during photosynthesis in the photic zone. During DIN (dissolved inorganic nitrogen) processing in PON (particulate organic nitrogen) the oxidation state changes from +3 (NO_2^-) or +5 (NO_3^-) to -3 (organic nitrogen) by intracellular reduction (Gianguzza, 2000). Therefore, by energy reasons, ammonium, NH_4^+, which contains nitrogen in -3 oxidation state, is preferentially consumed by phytoplankton. Multiple oxidation states of nitrogen in the marine environment gives rise to a large number of compounds involved in redox reactions that transform one species into another, associated with assimilation or disassimilation functions of marine organisms (Table 2.1).

Table 2.1 - The most important biologically influenced processes which transform nitrogen into seawater (after Gruber)

Process	**Organism**	**Redox environment**	**Biochemical role for the organisms**
NO_3^- Assimilation	Phytoplankton	aerobe	N source
NO_2^- Assimilation	Phytoplankton	aerobe	N source
NH_4^+ Assimilation	Phytoplankton	aerobe	N source
NH_4^+ Oxidation (Nitrification stage)	NH_4^+ oxidizers *e.g.Nitrosomonas*	aerobe	Energy source
NO_2^-Oxidation (Nitrification stage)	NO_2^- oxidizers *e.g.Nitrobacter*	aerobe	Energy source
N_2 Fixation	Cianobacteria etc. *e.g.Trichodesmium*	aerobe	N source
Ammonification	Bacteria/zooplankton	aerobe/anaerobe	N source
Denitrification	Bacteria	anaerobe	Electrons acceptor
Anammox (**An**aerobe**Amm**onium**Ox**idation)	Bacteria	anaerobe	Energy source

3. NW Black Sea inorganic nitrogen forms distribution (R/V „*Mare Nigrum*” expedition – April 2008)

Anoxic waters are characterized by a particular chemistry. Three important characteristics of this water type were mentioned by Richards (1965): (1) denitrification subsequently nitrates and nitrites losses, with the disappearance of oxygen, (2) reduction of sulfate ions, SO_4^{2-} and hydrogen sulfide, H_2S production, (3) redox potential decreasing thus reduced removal of organic matter (Horne, 1969).

The Black Sea, the largest permanently anoxic basin (Murray, 1991), provides an excellent environment for investigating the processes occurring in anoxic basins, especially for oxic-anoxic interface (Codispoti, 1991), and hence to investigate the distribution of inorganic nitrogen forms. Anoxic environment creates phenomena (Table 3.2.1) which determine different profiles of nitrates, nitrites and ammonium in the water column.

Nitrates profile (Fig. 3.1) highlights the Danube input confirmed by the highest value, 49.67µM (Station 1 – surface layer). Another high value was found in Station 2 – surface layer, 28.67 µM. Actually, we can observe two water bodies, a coastal one with nitrates concentrations in the range 5.08 - 49.67 µM (Station 1 and 2, maximum depth 50 m) and another one, a marine water body with nitrates concentrations within 0.24 - 9.79µM (Station 3 - 9). Statistical analysis (t-test) shows de difference between nitrates concentrations in the two water bodies extremely statistically significant ($p<0.0001$, $t = 4.53$, $df = 79$).

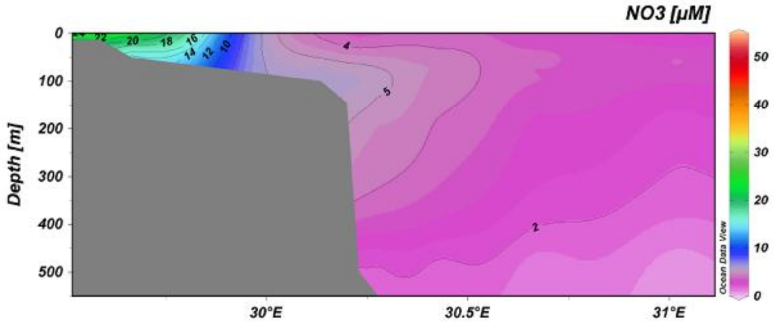

Fig. 3.1 - Nitrates vertical profile,
R/V "*Mare Nigrum*" expedition - April 2008

Minimum nitrates value, 0.24µM, is found at 500m depth, Station 9, noting decreased concentrations with oxygen disappearance, following denitrification (1) (Horne, 1969):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)+84.8HNO_3 \rightarrow 106CO_2+42.2N_2+148.4H_2O+16NH_3+H_3PO_4 \quad (1)$$

Intermediate forms of nitrogen cycle both in the nitrification and denitrification stages, nitrites recorded values between 0.01 - 1.36µM (Fig. 3.2). Like nitrates, the maximum value is found in the coastal stations area.

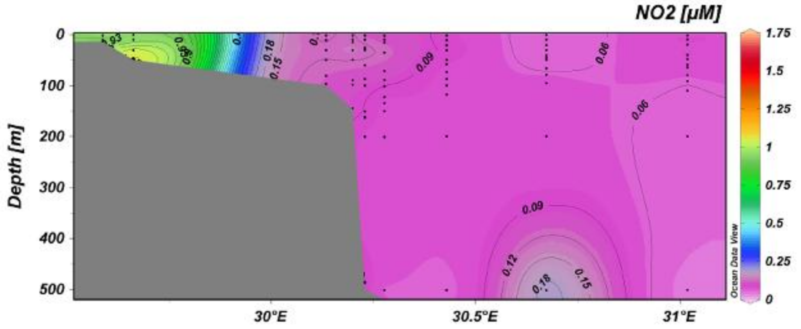

Fig.3.2 - Nitrites vertical profile,
R/V "*Mare Nigrum*" expedition– April, 2008

For marine waters, concentrations oscillated in the "*undetectable*" - 0.32 µM narrow range. Generally, nitrites profile had two maxima: one in the oxic layer due to nitrification processes and another one in the anoxic layer, Stations 5 - 9, following the same denitrification process (Fig. 3.2).

Ammonium had values within 0.87 - 48.53µM. As a response to decreased dissolved oxygen concentrations, ammonium is the dominant form of inorganic nitrogen at depths exceeding 200m.

The vertical distribution (Fig. 3.3) highlights the maximum at 500m depths, in anoxic waters, where predominates remineralization of organic nitrogen (constituent of proteins, polypeptides, amino acids), denitrification and sulfate reduction (2) contributing to marine nitrogen cycle.

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53SO_4^{2-} \rightarrow 106CO_2 + 53S^{2-} + 16NH_3 + 106H_2O + H_3PO_4 \tag{2}$$

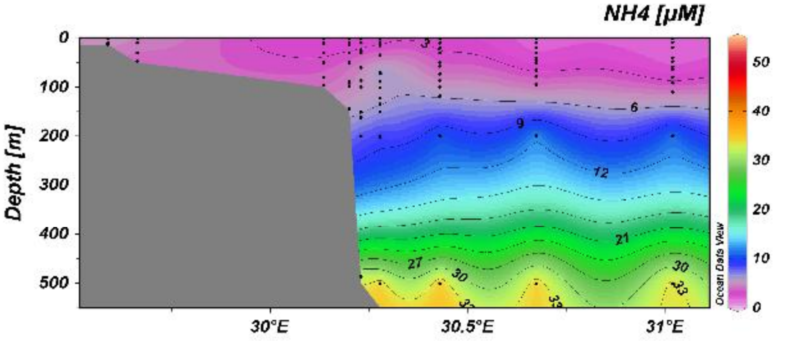

Fig. 3.3 - Ammonium vertical profile, R/V "*Mare Nigrum*"
expedition – April, 2008

Observing the inorganic nitrogen forms profile (nitrates, nitrites and ammonium) in the open sea (Stations 5 - 9) with bottom depths ranging from 500 – 2200m (Fig. 3.4), highlights the oxic, suboxic and anoxic layers.

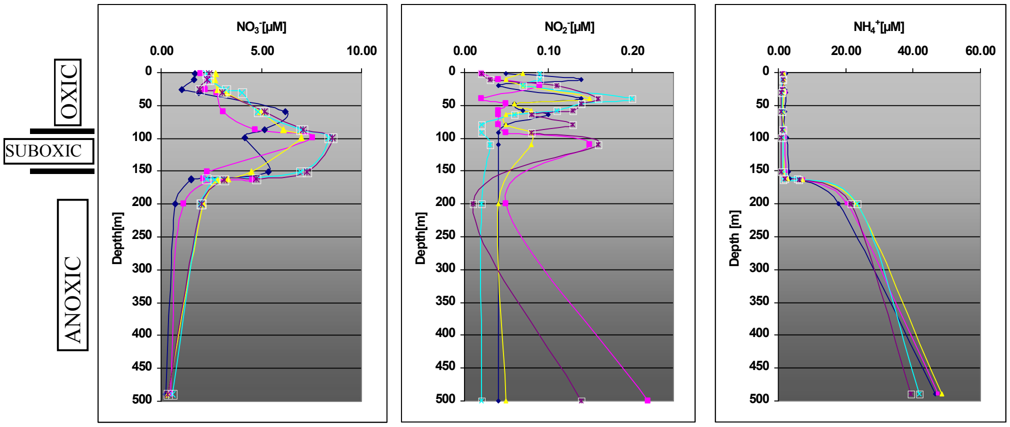

Fig. 3.4 - Nitrates, nitrites and ammonium vertical profiles, open sea stations (5-9) –R/V "*Mare Nigrum*" expedition – April 2008

In the oxic layer we observe the continuously increasing of nitrates, variability of nitrites and lowest values of ammonium concentrations. In the suboxic layer there were recorded maximum values for both nitrates with an average of 7.53µM (60 – 100m depth layer) (Codispoti, 1991) and nitrites (average 0.08µM), minimum for ammonium concentrations (average 1.61µM), and the anoxic layer (over 150m depth) where the latter prevails with an average concentration 33.06µM. Note minimum levels of nitrate and ammonium concentrations in the same suboxic area (aprox. 150m depth). These gradients imply that both species of nitrogen were consumed, without ammonium uptake from the anoxic zone (Codispoti, 1991).

With variations in "undetectable" - 8.37µM range, phosphates had two maxima: one in the oxic-suboxic area (50-150m) (Fig. 3.5) through regenerative and respiratory processes (Codispoti, 1991) and another one in the anoxic area, due to specific reactions such as: denitrification (1) and sulfate reduction (2).

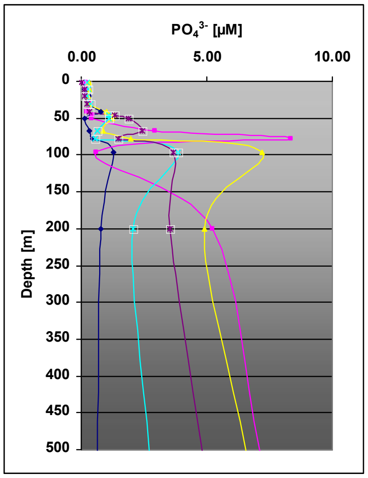

Fig. 3.5 - Vertical profile of phosphates –R/V"*Mare Nigrum*" expedition –April 2008

At the distance of the euphotic zone, biogeochemical cycles of N and P are primarily affected by organic matter mineralization and also denitrification and mineralization processes of rich nitrogen organic matter. Given the importance of knowledge concerning nitrogen species transformations in the biogeochemical cycle a parameter N * (Gruber and Sarmiento, 1997) was proposed to study the denitrification and nitrogen fixation distribution in the NW Black Sea waters. To simplify, the entire process of remineralization was considered as nitrification (oxidation to NO_3^-) even if the first step is ammonification. Parameter N * (μM) is quasi-conservative and combines linear nitrates and phosphates concentrations to study denitrification and nitrogen fixation distribution. It was calculated using the formula:

$$N^* = (N\text{-}16P + 2,\ 9\mu mol/kg)*0,\ 87\ [\mu M]$$

where N is the nitrate concentration (μM), P the phosphate concentration (μM) and 16 represents the stoichiometric ratio N:P (Redfield), constant during organic matter remineralization (Gruber and Sarmiento, 1997). The other constants were determined by the authors based on data collected from the entire World Ocean in the parameter design. This linear combination largely eliminates the effect of nitrification through nitrate and phosphate so N* variability is primarily due to denitrification and nitrogen fixation effects.

N * absolute value is arbitrary. Positive values can not be directly associated with nitrogen fixation or negative values with denitrification. Only changes that lead to a nonconservative behavior can be interpreted as denitrification or nitrogen fixation net effect (Gruber, 1997).

According to our nitrate and phosphate concentration measurements, N* has values between -112 ÷ 28μM, with average -10 μM and standard deviation 27μM. Our data revealed a high correlation between N* and salinity at the surface ($R^2 = 0.8637$) - the parameter is conservative. Together with increasing depth and lack of oxygen, N* becomes less correlated with salinity, the decreased coefficient of linear regression ($R^2 = 0.4492$) deviations could explain denitrification (Fig. 3.6).

N* vertical profile for open sea stations (5-9) has a minimum value at the suboxic/anoxic interface (about 160m) (Fig. 3.7), which coincides with the inflection points of the nitrates (lower concentrations) and ammonium (increased levels). Variability of N * reflects the difference between sources and losses of nitrogen due to denitrification-nitrogen fixing. Its internal distribution is affected only by phenomena as: transport, nitrification, denitrification (decreased ratio N: P), nitrogen fixation (increased ratio N: P) (Gruber, 1997). Given the stagnant waters of the Black Sea, N* variability highlights nitrogen cycle phases: nitrogen fixation nitrogen (positive values) and denitrification (negative values). An overview of values suggests that the denitrification intensity is much higher so that the area studied may be considered nitrogen sink. Hence, nitrate losses by denitrification in the anoxic zone are evident (Codispoti, 1991).

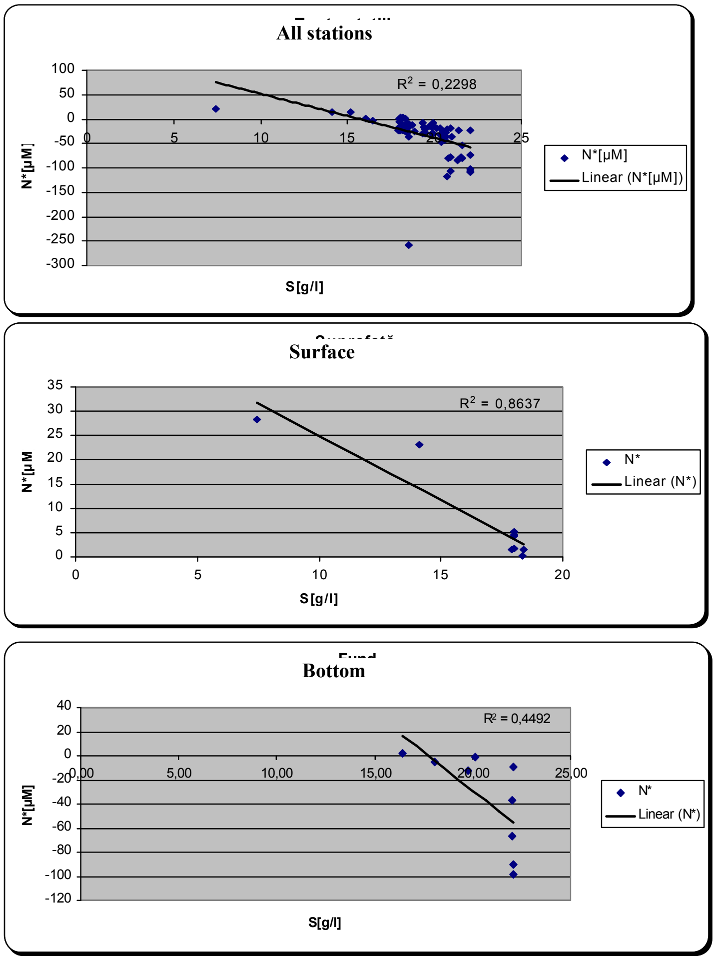

Fig. 3.6 - Linear correlations between N* and salinity – R/V “*Mare Nigrum*”, April 2008

N * distribution (Fig. 3.8) is variable depending on the analyzed water bodies. Thus, in the coastal Stations 1 - 4, with a significant contribution of nitrates, the parameter varies in the range -10 ÷ 28µM (average 6µM, standard deviation 8µM) due to high ratio N: P.

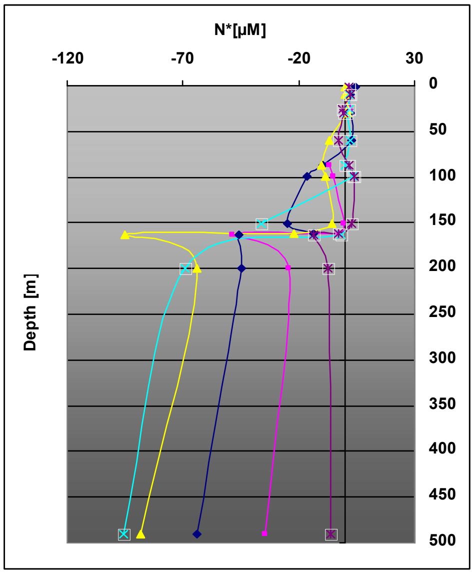

Fig. 3.7 - N* vertical profile, open sea stations,
R/V “*Mare Nigrum*” expedition – April 2008

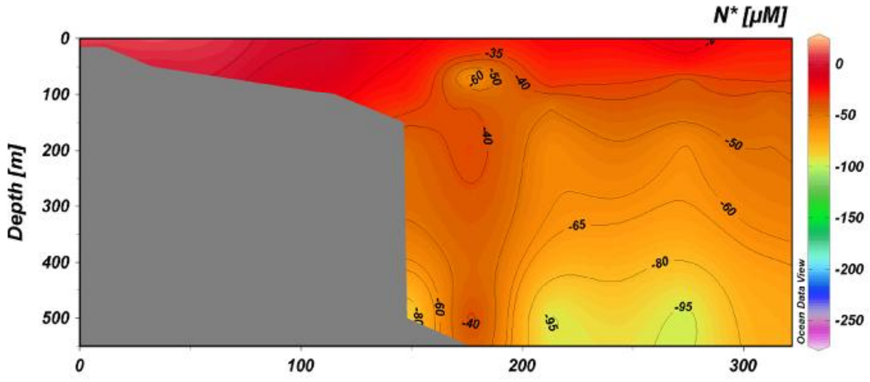

Fig. 3.8 N* distribution, R/V “*Mare Nigrum*” expedition,
April 2008

In the open sea (Stations 5 - 9) the range is between -112 ÷ 5µM (average -16µM, standard deviation 29µM), due to river input of nitrogen, dissolved oxygen regime and the dominance of redox processes of nitrogen cycle.

CONCLUSIONS

The results of our researches carried out in NW Black Sea in the spring of the year 2008 confirm that the values of general environmental indicators (temperature, oxygen, salinity) correspond to the specific seasonal characteristics known for the coastal and marine waters in NW Black Sea:

- nitrogen inorganic forms highlight the influence of the Danube input and the dominance of nitrate;
- inshore vertical distribution of nitrogen inorganic forms shows specific values for shallow areas with major river input where biological activity is intense;
- the same parameters in the offshore waters had characteristic values for stratified anoxic zones, where redox processes influence nitrogen cycle and ammonium is the dominant form;
- in the offshore waters nitrogen is the limiting factor of primary production;
- data provided by the parameter N*, tested as a new method for the Black Sea, prove to be useful in initiating the assessment process of the nitrogen role in the marine biogeochemical cycle;
- considering that globally 30-50% of the total nitrogen catch in the ocean is in oxygen minimum zones due to denitrification (Gruber, 1997), it is necessary to correlate these data with microbiology studies allowing quantification of microbial oxidation-reduction processes of nitrogen in the studied area.

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