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Report on the Impact of Nutrients on Shallow Water Ecosystems



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Acronyms

SCOR-UNESCO	Scientific Committee on Oceanic Research United Nations Educational, Scientific and Cultural Organization
WoRMS	World Register of Marine Species
CECIL CE-2020 UV-VIS	A model of spectrophotometer
Shimadzu UV-1900i UV	A model of spectrophotometer
UV-VIS	Ultraviolet-visible spectroscopy
Std.Dev / SD	Standard deviation
PSU	Practical Salinity Unit

Executive summary

This Report has been prepared as part of the ANEMONE Project "Assessing the vulnerability of the Black Sea marine ecosystem to human pressures" funded through the Joint Operational Programme Black Sea Basin 2014-2020.

The report on the impact of nutrients on shallow waters ecosystem, represents a study case on phytoplankton composition conducted based on historical and new data with the aim of analyzing the impact of the nutrients useful to provide information as decision support to assess the potential future development and the nutrients input in the Romanian coastal zone.

The report conclusion highlighted that the seasonal cycle of phytoplankton assemblages were linked to seasonal variations in Danube's discharges, salinity, temperature and nutrients regime. In Mamaia Bay, according to the seasonal cycle of hydrographic conditions, phytoplankton showed two main patterns related to two main events: Danube's discharges and temperature. The effect of the nutrient input caused by the winter mixing and Danube's discharges was observed during late winter and spring, when phytoplankton density and biomass peaks were recorded.

High values of phytoplankton can occur both as a result of extreme climatic phenomena (Danube hydrological regime, temperature regime, wind, wave, current and precipitation regime) and anthropogenic influence that can temporarily destabilize the ecological state.

All these results from the report highlights the necessity and the importance of future research and the alignment to modern and efficient equipment.

1 Introduction

Phytoplankton forms the basis of aquatic food chains and has global importance in the ecosystem's functioning. The dynamics of these photosynthetic organisms are related as a result of the annual temperature and salinity fluctuations, the light and nutrient availability and the mixing in the water column. Climatic conditions can influence these environmental factors and can change the structure of phytoplankton community, seasonal dynamics and taxonomic composition favoring species with traits best adapted to changing conditions associated with climate change (Winder & Sommer, 2012). Understanding the factors that control phytoplankton composition and dynamics is important for estimating the impact of nutrients and climatic conditions on aquatic ecosystems.

Besides phytoplankton structure and abundance, chlorophyll a concentrations in seawater samples was used to estimate the biomass and the photosynthetic capacity of phytoplankton (SCOR-UNESCO, 1966).

2 Material and Methods

The annual cycle of the phytoplankton community in the shallow waters of Mamaia Bay was analysed based on:

- 101 phytoplankton samples from 2018;
- 94 chlorophyll *a* samples from 2018;
- NIMRD phytoplankton and physico-chemical parameters of Mamaia Bay long term dataset (1959/1976/1980-2018)
- 208 samples for physico-chemical parameters from 2018;
- Daily Danube's discharges retrieved from the National Institute of Hydrology and Water Management website (http://www.inhga.ro/diagnoza_si_prognoza_dunare) from 2018;
- 2006 Global Radiation data (Constanta station) retrieved from World Radiation Data Centre (http://wrdc.mgo.rssi.ru/).

The biological and physico-chemical samples were collected from the surface layer of the seawater (0.1-0.5 m) with a bucket and then transferred to labelled bottles (Moncheva, 2008).

Taxonomic composition and cell counts were done under an inverted microscope connected to a video-interactive image analysis system at 400x magnification using the Utermöhl (1958) method and counting chambers (Utermöhl chambers). The individual cell biovolume (V, μ m3) was derived through the approximation of the cell shape of each species to the most similar regular solid and calculated with the respective formulas used routinely in the lab. Cell biovolume was then converted to weight (W, ng) following Hatchinson (1967).

Species identification was performed accordingly to Schiller (1937), Kisselew (1950, 1951), Proshkina-Lavrenko (1955), Carmelo (1997) and the taxonomic nomenclature according to the online database of World Register of Marine Species (WoRMS).

Chlorophyll *a* was determined through the method based on the extraction of the pigment with 90% acetone (after separation on a cellulose filter) and the measurement of the absorbance of the sample at four wavelengths (λ = 750nm; λ = 630nm; λ = 645nm and λ = 663nm). The calculation of the chlorophyll concentrations were made according to the SCOR-UNESCO trichromatic equations:

$$c = \frac{(11.64 \times A_{663} - 2.16 \times A_{645} + 0.10 \times A_{630}) \times v}{v} \mu g/l,$$

where: 11.64; 2.16; 0.10 - molar extinction coefficients

v - volume of extract in acetone 90%

V - the volume of the seawater sample used.

Additionally, we tested the differences between two instruments used to analyze a set of 29 daily samples from 2021. In order to identify if there were significant differences due to the instrument used, which could create errors in the long-term chlorophyll *a* dataset, a t-test was performed.

The samples were first analyzed using a more than 20 years old CECIL CE-2020 UV-VIS spectrophotometer. Then, the same set of samples were analyzed using a new generation double-beam UV-VIS spectrophotometer (Shimadzu UV-1900i UV), designed to enhance usability, performance and data compliance. t-tests were performed to compare the two spectrophotometers' results regarding the final chlorophyll *a* concentration and the absorbances measured at the four different wavelengths.

3 Results and discussions

3.1 Nutrient trends at Mamaia Bay

In the period of 1959-2018, the annual mean phosphate concentrations $(PO_4)_{3^-}$ varied between 0.13µM (1967) and 12.44µM (1987) and a decrease has been observed since 1987.

In 2018, the average phosphate concentrations calculated at 0.46 μ M exceeded the range characteristic for the reference period of the '60s (multiannual average 1959-1969 0.28 μ M ± 0.14 μ M) (Figure 3.1).

Long-term, 2018 monthly means were significantly different (t-test, interval of confidence 95%, p=0.0001, t=9.3184, df=22, Std.Dev. of diff.=0.222). However, 2018 monthly means were significantly higher than those of the reference period (1959-1969).



Figure 3.1 - Comparative status of monthly multiannual averages of phosphate concentrations in seawater- Mamaia Bay between 1959-2017 and 2018

Nitrate - Multiannual (1976-2017) and 2018 monthly means are comparable (t-test, interval of confidence 95%, p=0.9914, t=0.0109, df=22, Std.Dev. of diff.=1.305) due to the high values of Nitrate from 2018. Long-term (1976-2018), the annual average in 2018 was 7.30 μ M (Figure 3.2).



Figure 3.2 - Comparative status of monthly multiannual averages of nitrate concentrations in seawater-Mamaia Bay between 1976-2017 and 2018

Nitrite - Multiannual (1976-2017) and 2018 monthly means are significantly different (t-test, coverage 95%, p=0.0003, t=4.3452, df=22, Std. Dev. of diff.=0.069) due to the diminished ones of 2018. Long-term (1976-2018), the average concentration of 2018 was 0.53μ M (Figure 3.3).



Figure 3.3 - Comparative status of monthly multiannual averages of nitrite concentrations in seawater -Mamaia Bay between 1976 - 2017 and 2018

Ammonium - Multiannual (1980-2017) and 2018 monthly means are not significantly different (t test, coverage 95%, p=0.07751, t=1.8519, df=22, Std.Dev. of diff=0.783). Long-term (1980-2018), the annual average of 2018 was 4.85μ M (Figure 3.4).



Figure 3.4 - Comparative status of monthly multiannual averages of ammonium concentrations in seawater - Mamaia Bay between 1976 - 2017 and 2018

The high averages of May and November were due to the periods of mineralisation of organic substances produced as a result of algal blooms and the prolonged upwelling phenomenon that occurred between 22.10 to 12.11.2018.

Silicate - Multiannual (1980-2017) and 2018 monthly means are not significantly different (*t*-test, coverage 95%, p=0.1313, t=1.5673, df=22, Std.Dev. of diff=2.722). Annual means oscillated from 6.7 μ M (1993) to 66.3 μ M (1972), with an average of 19.1 μ M in 2018, representing 54% of the multiannual mean of the reference period 1959-1969 (35.1 μ M) (Figure 3.5).



Figure 3.5 - Comparative status of monthly multiannual averages of seawater silicate concentrations -Mamaia Bay between 1959 - 2017 and 2018

3.2 Phytoplankton taxonomic composition

In 2018, 160 phytoplankton species from 12 taxonomic classes were identified in the study area. A relatively high diversity of the phytoplankton assemblages was recorded in the shallow waters of Mamaia Bay, supporting the increasing trend over the last 20 years (R^2 =0.62) (Figure 3.6).



Figure 3.6 - Multiannual evolution of phytoplankton diversity in the shallow water station from Mamaia Bay

The diatoms and the dinoflagellates identified in 2018 represented 66% of the total number of species identified in 2018, followed by chlorophytes (12%) and cyanobacteria (9%). The other classes represented the other 13%, having up to 3 species each (Figure 3.7).

Among diatoms (59 species), genera Chaetoceros (15), Thalassiosira (7), genera Nitzschia (5), Synedra (3), Coscinodiscus (2) and Achnantes (2) showed the highest species richness. The highest richness of the diatoms were observed in January (34) and November (32). Their numbers dropped to 14-16 species during July-August, when the water temperature reached its maximum (25-26 $^{\circ}$ C). The lowest diversity was found in December, probably due to daylight duration and intensity, besides temperature.

The dinoflagellates were mainly represented by the genera Protoperidinium (8 species), Prorocentrum (5), Gyrodinium (5) and Gymnodinium (4). The highest number was recorded in October (29) and May-June (24-25).

A relatively high number of species were identified for Chlorophyceae (20 species) and Cyanophyceae (15), the highest richness being found in April-May (13 and 12 respectively). The other classes were represented by a few species, such as: Prymnesiophyceae (3 species), Cryptophyceae (4), Dictyochophyceae (3) and Trebouxiophyceae (2) (Figure 3.7).



Figure 3.7 - Phytoplankton taxonomic composition during 2018

Based on the ecological composition of the Mamaia Bay phytoplankton, 69% of the species identified were marine. The freshwater phytoplankton represented up to 40%, the highest proportion coinciding with the highest value of Danube's discharge in April and lower salinity values from May (12 PSU) (Figure 3.8).



Figure 3.8 - Ecological composition of the Mamaia Bay phytoplankton in 2018

In 2018, the highest diversity was observed in spring, with 118 species compared to the other seasons where the number slightly decreased (103 in summer, 100 in autumn and 97 in winter) (Figure 3.9). The spring biodiversity followed the common taxonomic pattern with the majority represented by the diatoms (32%) and dinoflagellates (27%) present in the community, but also a high contribution of freshwater species (chlorophytes -16% and cyanobacteria - 12%), which are primarily linked to the conditions accompanied by higher Danube's discharges.

During summer, the community slightly changed in terms of the assemblage, where diatoms remained the same (32%) while the dinoflagellates (33%) increased. It should be taken into consideration that during summer, many small flagellates develop. However, they are difficult to identify both in terms of the scientist skill and methods used (fixation, time until microscopic processing, concentration method). During autumn, the contribution of diatoms (39%) and dinoflagellates (32%) returns in the favor of the diatoms, a ratio that increased during winter (Figure 3.9).



Figure 3.9 - Seasonal variation of the phytoplankton taxonomic composition at Mamaia Bay, in 2018

3.3 Quantitative analysis of phytoplankton

Total phytoplankton ranged from 22·103 cells/L to 24·106 cells/L and from 0.01 g/m3 to 5.38 g/m3. The distribution of phytoplankton quantities showed a common variability between seasons with the highest phytoplankton development registered in spring. The second peak in biomass was recorded during autumn when dinoflagellates and large diatoms usually make up the bulk of the assemblages (Figure 3.10).

The lowest densities (up to $26 \cdot 103$ cells/L) were recorded during winter and summer months, when the temperature and light intensities are at the extremities of the phytoplankton tolerance range.



Figure 3.10 - Seasonal variation of total phytoplankton abundance and biomass at Mamaia Bay, in 2018

The monthly average abundance ranged between $49 \cdot 103$ cells/L and $9.6 \cdot 106$ cells/L and the average biomass between 0.04 g/m^3 and 2.00 g/m3. $89 \cdot 91\%$ of the maximum means recorded in 2018 were due to the development of diatoms (particularly *Skeletonema costatum*) which reached the exponential growth phase during April. The lowest mean biomass and density were reported in winter, when the community was dominated by non-diatoms (mainly cryptophytes, dinoflagellates and cyanobacteria) (Figure 3.11).



Figure 3.11 - Monthly variation of the phytoplankton average abundance and biomass at Mamaia Bay, in 2018

The phytoplankton community in the winter months (especially January and February) was characterized by the dominance of cyanobacteria in average density (46%-52%) and dinoflagellates (33%-54%) and diatoms (25%-54%) in average biomass. This cold season assemblage continued its development till the beginning of spring (March) (Figure 3.12). This fact corresponded to a relatively high salinity (16 PSU), increasing discharges (10100 m3/s), a low temperature (4.2° C) and an increasing global radiation (1262 J/cm2, average for March 2006) (Figure 3.13, Figure 3.14).



Figure 3.12 - Monthly phytoplankton taxonomic structure based on average abundance and biomass

The phytoplankton community of the next three months (April, May and June) was mainly composed of diatoms (47%-91% in average density and 70%-89% in average biomass), joined by a higher proportion of cyanophytes (21%-35%) and cryptophytes (7%-18%) during May and June. In terms of biomass, the dinoflagellates reached a higher contribution in June (24%) and increased during the next four months to 29%-91%, recording their maximum in September.

In terms of density, the phytoplankton assemblages from these warm season months (July-October) were mainly composed of cyanophytes (35%-48%), diatoms (18%-58%) and cryptophytes (10%-32%).



Figure 3.13 - Monthly average variation of global radiation at Constanta station during 2006 (according to World Radiation Data Centre)



Figure 3.14 - Monthly average variation of temperature, salinity and Danube's discharge during 2018

3.4 Blooms

In 2018, bloom events (over 106 cells/L) occurred every season in Mamaia Bay, but only the spring conditions maintained the most prolonged and intense event.

The intensity of the winter bloom was low (up to 2.106 cells/L). In 6 out of 20 samples collected during winter, the total phytoplankton community reached bloom densities due to a multispecies assemblage formed mainly by diatoms and cyanobacteria, such as: *Pseudanabaena limnetica*, *Limnolyngbya circumcreta*, *Skeletonema costatum*, *Thalassiosira nordenskioldii* and *Diatoma tenuis*. When this assemblage developed at the end of January, it coincided with a slight increase in the Danube's discharge from 7500 m³/s to 7900-8070 m³/s.

The intensity of summer blooms slightly increased compared to those recorded during winter and autumn. In 5 out of 30 samples, multispecies assemblages reached 106-4·106 cells/L. In the summer season, the cryptophytes (*Hillea fusiformis* and *Komma caudata*) joined the community dominated by cyanobacteria (*Pseudanabaena limnetica*, *Snowella lacustris*, *Merismopedia tenuissima*, *Limnolynbya circumcreta*) and diatoms (*Cyclotella caspia* and *C. meneghiniana*). The summer blooms from the end of July and the beginning of August occurred in high temperature (26-27°C) and low salinity (10-14 PSU) conditions.

During autumn, only one blooming event was registered. The multispecies assemblage was mainly represented by diatoms (*Cerataulina pelagica*, *Thalasiossira subsalina*, *Leptocylindrus danicus*, *L. minimus*, *Skeletonema costatum*) and the cyanobacteria *P. limnetica*.

The spring bloom dominated the annual cycle of phytoplankton abundance. The improving light condition is considered to be the main factor for triggering spring blooms (Rumyantseva, 2019). Another factor would be the highest Danube's discharges ($11000-15000 \text{ m}^3$ /s) from 2018. Thereby, 21 out of 34 samples collected during spring recorded bloom densities, from which 48% were between 1·106 -5·106 cells/L, 33% were between 10·106 -20·106 cells/L and the other were between 5·106 - 10·106 cells/L. The spring blooms began with the ample development of the cyanobacteria *Pseudanabaena limnetica* (2·106 -4·106 cells/L) at the end of March and the beginning of April. It was then replaced by the annual spring cycle of the diatom S. *costatum*. While S. *costatum* accounted for over 90% of most of the samples from April, there were two occasions when P. limnetica managed to reached bloom densities (1·106 and 2·106 cells/L). Towards the end of spring, a low intensity bloom of the diatom *C. pelagica* emerged.

3.5 Chlorophyll *a* variation in Mamaia Bay during 2018

The chlorophyll *a* content determined in the shallow waters of Mamaia in 2018 varied between a lower range (0.64 μ g/L and 10.80 μ g/L) compared to the values recorded one year before (0.19 μ g/L and 19.03 μ g/L) and one year after (0.21 μ g/L and 15.18 μ g/L).

The maximum values were recorded during autumn. This ranged from 0.99 μ g/L to 10.80 μ g/L (November), with a seasonal average value of 3.55 μ g/L. The maximum of this season was correlated with high values of biomass of the species S. *costatum*, L. *minimus*, *Protoperidinium granii*, *Neoceratium furca*, *Lingulodinium polyedrum*, C. *pelagica* and *Prorocentrum micans* (2 g/m³ - 4.60 g/m³), with the last two species having the most considerable contribution (Figure 3.15).

During winter, lower values were registered, between 1.04 μ g/L and 7.27 μ g/L, with a seasonal average of 3.45 μ g/L. It should be noted that the sampling effort for this season was slightly reduced (19 samples, compared to 22-25 samples in the other seasons) due to meteorological conditions, which sometimes did not allow access to the sampling site.

Higher values were recorded during spring (up to $8.74 \mu g/L$ with a seasonal average of $3.34 \mu g/L$) and summer (up to $8.58 \mu g/L$ with a seasonal average of $3.05 \mu g/L$). The diatoms *C. pelagica* and *S. costatum* achieved the highest values of biomass during spring. At the start of summer, the continued development of *C. pelagica* was observed and its gradual replacement by *Cyclotella meneghiniana* and *Pseudosolenia calcar-avis* and by the dinoflagellates *P. granii, Akashiwo sanguinea, Oblea rotunda* and *P. micans*, with the latter registering the highest value.



Figure 3.15 - Variation of chlorophyll a (µg/L) and phytoplankton biomass in the shallow waters of Mamaia Bay, during 2018

3.5.1 Performing t-test

An unpaired *t*-test was conducted on the set of 29 chlorophyll *a* samples from January 2021 to compare both the chlorophyll *a* concentrations and the absorbance readings at every wavelength, obtained by using CECIL CE-2020 UV-VIS spectrophotometer and Shimadzu UV-1900i UV-VIS spectrophotometer.

3.5.2Chlorophyll *a* concentrations

The unpaired t-test conducted to compare the chlorophyll *a* concentrations obtained from Cecil and Shimadzu spectrophotometers showed that there was not a statistically significant difference in the results from Cecil (mean=1.95 and SD=2.36) and Shimadzu (mean=2.05 and SD=2.39), two-tailed P value = 0.8768 (Table 3.1).

Group	Cecil	Shimadzu		
Mean	1.95	2.05		
SD	2.36	2.39		
Ν	29	29		
	two-tailed P-value	0.8768		
Intermediate values	t	0.1557	0.1557	
	df	56	56	
	standard error of difference	0.624		

Table 3.1 - Statistical analysis of the chlorophyll a concentrations obtained by reading the	samples at
two spectrophotometers	

3.5.3 Absorbance readings

The unpaired t-test conducted to compare the absorbance reading at each wavelength for Cecil and Shimadzu spectrophotometers showed that there was not a statistically significant difference in the results for the absorbance readings (Table 3.2):

750 nm, Cecil (mean=0.0071 and SD=0.0047) and Shimadzu (mean=0.0067 and SD=0.0041),

two-tailed P value = 0.7215.

- 630 nm, Cecil (mean=0.0148 and SD=0.0094) and Shimadzu (mean=0.0146 and SD=0.0087), two-tailed P value = 0.9078.
- 645 nm, Cecil (mean=0.0141 and SD=0.0085) and Shimadzu (mean=0.0139 and SD=0.0080), two-tailed P value = 0.8994.
- 663 nm, Cecil (mean=0.0251 and SD=0.0220) and Shimadzu (mean=0.0256 and SD=0.0221), two-tailed P value = 0.9386.

Table 3.2 - Statistical analysis of the absorbance readings at two spectrophotometers, at eachwavelength used in the trichromatic equation for the chlorophyll a calculation

Wavelength		750 nm		630 nm		645 nm		663 nm	
Group	C *)	S*)	С	S	С	S	С	S	
Mean	0.0071	0.0067	0.0148	0.0146	0.0141	0.0139	0.0251	0.0256	
SD	0.0047	0.0041	0.0094	0.0087	0.0085	0.0080	0.0220	0.0221	
Ν	29	29	29	29	29	29	29	29	
	two-tailed P value	0.7215		0.9078		0.8994		0.9386	
Intermediate	t	0.3583	0.1163	0.1270	0.0774	t	0.3583	0.1163	0.1270
values	df	56	56	56	56	df	56	56	56
	standard error of difference	0.001	0.002	0.002	0.006	standard error of difference	0.001	0.002	0.002

*) C - Spectrophotometer Cecil

*) S - Spectrophotometer Shimadzu

4 Conclusions

During 2018 in Mamaia Bay, it was highlighted that the seasonal cycle of phytoplankton assemblages was linked to seasonal variations in Danube's discharges, salinity, temperature and nutrients regime. In Mamaia Bay, according to the seasonal cycle of hydrographic conditions, phytoplankton showed two main patterns related to two main events: Danube's discharges and temperature. The effect of the nutrient input caused by the winter mixing and Danube's discharges was observed during late winter and spring, when phytoplankton density and biomass peaks were recorded.

With the increase of the Danube's discharges, which registered the annual maximum in April, the nutritional intake (silicates) allowed for the bloom event of the diatom *Skeletonema costatum* from April to May. The bloom contributed to the increase of ammonium concentrations in May as a result of the decomposition of the organic matter produced photosynthetically.

There was not a statistically significant difference between Cecil and Shimadzu. Therefore, the new equipment can be used to continue the long-term datasets with accurate measurements and time efficiency.

High values can occur both as a result of extreme climatic phenomena (Danube hydrological regime, temperature regime, wind, wave, current and precipitation regime) and anthropogenic influence that can temporarily destabilize the ecological state.

All these results from this report highlights the necessity and the importance of future research and the alignment to modern and efficient equipment.

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