Ecological Status of Romanian Black Sea Waters According to the Planktonic Communities *(Laura Boicenco, Luminita Lazăr, Elena Bișinicu, Oana Vlas, George Harcotă, Elena Pantea, Cristina Tabarcea, Florin Timofte)*	"Cercetări Marine " Issue no. 49 Pages 34 - 56	2019

ECOLOGICAL STATUS OF ROMANIAN BLACK SEA WATERS ACCORDING TO THE PLANKTONIC COMMUNITIES

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ABSTRACT

This paper reports Romanian Black Sea waters' plankton diversity and abundance for three consecutive years (2015-2017). In these years, biodiversity has varied significantly in a seasonal aspect. Thus, we identified 199 species of phytoplankters, 25 microzooplankters, 23 mesozooplankters, and 3 gelatinous zooplankters. We also presentetd data on the microzooplankton community (tintinnids), two decades after being completely neglected in the monitoring of Romanian waters. The plankton diversity and abundance are crucial issues for the ecological status within Marine Strategy Framework Directive's (MSFD) descriptors and indicators. The ecological status was evaluated for cold and warm seasons. As a result, all water bodies from the Romanian littoral were in "good ecological status" (GES), according to phytoplankton and mesozooplankton status. Regarding coastal waters, the phytoplankton biomass exceeded the target value, defining this water body as "non-GES".
Key-Words: Plankton, Diversity, Density, Biomass, Environmental status

AIMS AND BACKGROUND

Twenty years, early (1970s – 1990s), the Black Sea ecosystem was threatened by various pressures, notably pollution, eutrophication, overfishing, introduction of exotic species, climate change and other human-related activities, resulted in large-scale ecological changes (Kideys, 2002), including a huge decrease in the area occupied by *Phyllophora* (a red seaweed), large areas of oxygen depletion and increased dominance of phytoplankton as primary producers in shallow shelf waters.

Finally, during the last decade of the past century, nutrient pollution in the Black Sea region decreased slightly because of implementation of best environmental practices in the agricultural sector in the EU member states

(Danube catchment area), economic recession in the former socialist countries, and the ecosystem begun to partially regain its pre-eutrophication features.

Because the marine environment is a precious heritage that must be protected, preserved and, where feasible, restored in order to maintain biodiversity, ecosystem functioning and to ensure clean, healthy and productive seas, two normative acts of the European Union – Water Framework Directive (2000) (WFD), and the more recent EU Marine Strategy Framework Directive (2008) (MSFD) require Member States to take all necessary measures for conservation, maintenance, sustainable use, and/or improvement of biodiversity of Europe's seas.

Referring to the Black Sea pelagic habitats and to reflect Good Environmental Status proposed by MSFD, they must be able to provide ecosystem services (biodiversity, carbon cycling, food provision through supporting marine food webs). Thus, to monitor the state of this compartment of the Black Sea ecosystem, and to assess its environmental status in terms of the related MSFD's indicators is necessary and this is what we planned to present in this paper.

Phytoplankton is one of the basic biological quality elements in the Water Framework Directive (WFD) and of great concern in four MSFD's descriptors: Biodiversity (D1), Non-Indigenous Species (D2), Fodder chain (D4) and Eutrophication (D5) (Commission Decision EU 2017/848).

Resuming the study of the microzooplankton component (in particular of the Tintinnid community), after a period of more than a decade allowed to highlight some changes in their composition, characterised by the evidence of the penetration of new non-indigenous species. Thus, the microzooplankton component responds to Descriptor D2 (criteria D2C1 and D2C3).

Mesozooplankton indicators can provide valuable information on the status and distribution of pelagic habitats for D1 and *Noctiluca scintillans* for D5.

Knowledge of the abundance and biomass fluctuations of gelatinous zooplankton is necessary to understand the dynamics of the pelagic subsystem and the pressure it is subjected to, many of these species feed on zooplankton eggs and fish larvae (Harcota et al., 2018). Monitoring the abundance of gelatinous species is required in two descriptors – D1 and D2 (in the circumstances in which two ctenophores living now in the Black Sea are non-indigenous).

EXPERIMENTAL

We collected samples of water, phytoplankton, microzooplankton, mesozooplankton and gelatinous zooplankton, between 2015 and 2017, during six cruises, from 13 profiles distributed in coastal (CWB), transitional (TWB) and marine bodies (MWB) of the Romanian Black Sea waters (Fig.1). The samples of water were collected from the surface layer (0-10m) with Niskin bottles - volume 5L (without rosette). Samples are stored in labelled plastic containers in freezer bags. Water samples for the determination of dissolved oxygen were taken in special bottles, Winkler. Other parameters (temperature, salinity, transparency) were measured in situ with CTD and Secchi disk. Dissolved oxygen was determined by Winkler method (SR EN 25813:2000) according to Grasshoff, 1999. Nutrients were analysed spectrophotometrically (Grasshoff, 1999; Mullin & Riley, 1955).

Chlorophyll *a* was determined by extraction with acetone 90% (Millipore cellulose filter, 0.45 µm) and measuring absorbance of the sample at three wavelengths (Λ = 630nm; Λ = 645nm and Λ = 663nm). The calculation of chlorophyll *a* concentration is by SCOR-UNESCO (1966) tri-chromatic equations.

The samples of phyto- and microzooplankton, in a volume of 500 ml, were taken from standard depth, using NANSEN bottles and stored in labelled glass containers, then preserved in formaldehyde 37%. The phytoplankton samples were processed by the sedimentation method (Morozova-Vodianitkaia, 1948; Utermöhl, 1958; Bodeanu, 1987 - 1988). The taxonomic identification of species and counting of cells in a 0.1 ml fraction of each sample was carried out in Utermöhl counting chamber under Olympus inverted microscope, using a 40x objective lens for nanoplankton (less than 15–20µm) and 10x or 20x for larger cells. The cell biovolume was calculated using relevant morphometric measurements of phytoplankton cells and approximation by corresponding geometric shapes after (Edler, 1979), (Hillebrand et al., 1999).

Microzooplankton samples were concentrated to a final volume of 10 ml by repeated sedimentations. The final volume was fully analysed on the inverted microscope (Olympus XI 51), with a magnification factor of 200x and 400x.

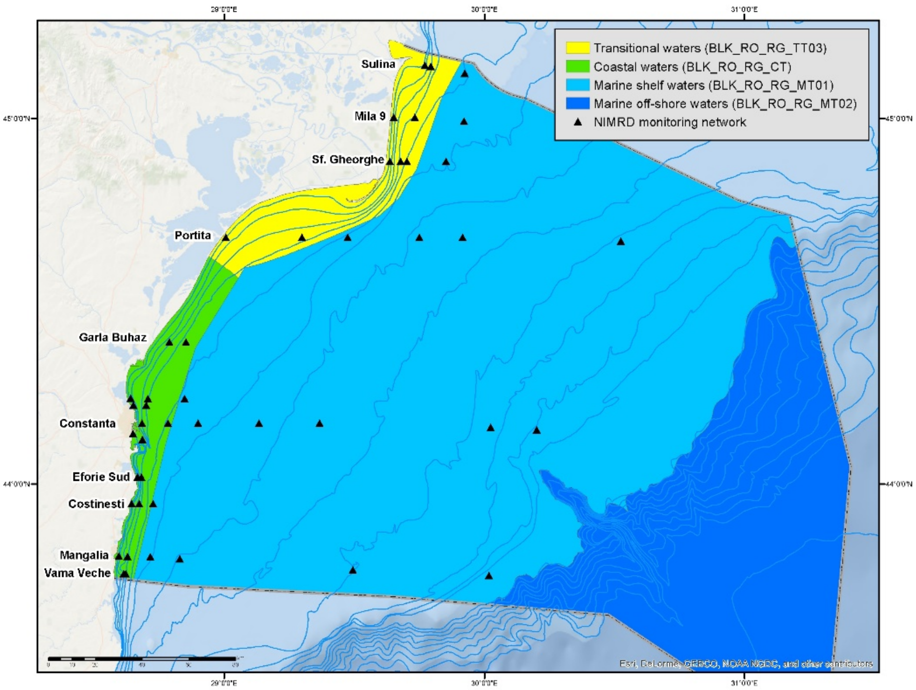

Fig. 1. Network of water and plankton sampling stations, 2015-2017.

Collecting of mesozoplankton samples was performed using a JUDAY net (36 cm diameter, 150 μm mesh size). Samples were collected by vertical hauls, stored in 500 ml plastic jars, and preserved with 4% buffered formaldehyde solution. The collected samples were further analysed under the Olympus SZ 61 stereo microscope.

The gelatinous zooplankton sampling was carried out with the help of the HANSEN net, and by vertical hauls on the whole water column, and the counting and measuring of the individuals was done aboard the ship.

In order to characterize the marine environmental status from the point of view of the planktonic communities, the applied indicators were those from the classification system developed during the MSFD reporting cycles (Boicenco et al., 2018) for the Romanian Black Sea waters, namely phytoplankton biomass, mesozooplankton, copepods and *Noctiluca scintillans* biomass, number of non-indigenous species and *Mnemiopsis leidyi* biomass.

RESULTS AND DISCUSSION

WATER QUALITY

It was expected beforehand to find minimum seawater temperature in early spring. Accordingly, the lowest value was measured in March 2017 (Est Constanta 1 (10 m) while its peak was found in summer, August 2016 (Est

Constanta 2, surface) (Table 1). Although a high variability expressed as interquartile range (IQR) was found, no uncharacteristic values were identified in the dataset. In contrast, salinity had four abnormal values below 4.78 psu (Grubbs test), all of them from June 2015 and caused by the excessive Danube's flow, approximately 30% above the average discharge flow and the discharge of Eforie Sud wastewater treatment plant. Thus, minimum salinity was measured at Sulina 10m (0m) and maximum, offshore, at Est Constanta 6 (0m) in November 2017 being a normal value for the Black Sea (Konovalov and Murray, 2001). The surface layer (0-10m) was well oxygenated because of the permanent exchange with the atmosphere and photosynthetic production. Based on the values' distribution, we found six abnormal values, two less than 246.6 µM and four greater than 416.7 µM. All unusual values belong to summer 2015 (June). Thus, peaks were found in the entire water column (Portita 20m) because of diatoms bloom ($3.36 \cdot 10^6$ cells/L, 0m and $2.94 \cdot 10^6$ cells/L, 10m). In the same stations, chlorophyll *a* has reached extreme concentrations, 33.82 µg/L and 17.30 µg/L respectively.

Table 1. Descriptive statistics of physico-chemical parameters, Romanian Black Sea waters, 2015-2017

Variable	N	Mean	Median	Min.	Max.	25^{th} percentile	75^{th} percentile	Std. Dev.
T, °C	196	15.28	12.81	6.03	25.35	8.56	22.53	6.67
S, psu	196	15.81	16.34	0.19	19.75	14.37	18.14	3.27
O_2, µM	196	328.3	327.3	211.3	452.7	303.3	350.8	35.8
PO_4, µM	198	0.23	0.12	0.01	2.71	0.06	0.27	0.34
SiO_4, µM	198	21.7	7.0	1.3	395.2	3.5	17.8	46.8
NO_3, µM	198	4.24	2.48	0.01	52.00	1.60	4.40	5.37
NO_2, µM	198	1.37	0.32	0.03	50.85	0.11	0.79	4.45
NH_4, µM	198	5.73	4.19	0.21	26.62	1.40	8.30	5.47
DIN*, µM	198	11.34	9.46	1.65	104.09	4.87	14.36	10.31

*DIN-dissolved inorganic nitrogen (sum of NO_3, NO_2 and NH_4)

Among the nutrients, high values of phosphate and silicate were clearly widespread in the vicinity of the Danube's mouths. Thus, the maximum phosphate concentration was from April 2016 (Sf. Gheorghe 5M, surface) (Fig. 2). Despite the lack of correlation with salinity, the peak is most probably due to excessive Danube's flow from March-April 2016. Silicate recorded maximum in March 2017, in the water column Est Constanta 5. Due to low chlorophyll *a* level (0.81 µg/L) we assume that the silicate concentrations were measured after diatoms bloom. From the inorganic nitrogen forms, nitrate and nitrite were dominant at the Danube's mouths while ammonium surface distribution suggests as a source either other rivers from the N-NW region or water masses circulation.

Generally, the significant influence on chlorophyll *a* concentration derived from salinity (r=-0.64), nitrate (r=0.55) and nitrite (r=0.53). The seawater temperature significantly supports the dinoflagellates density progress (r=0.53).

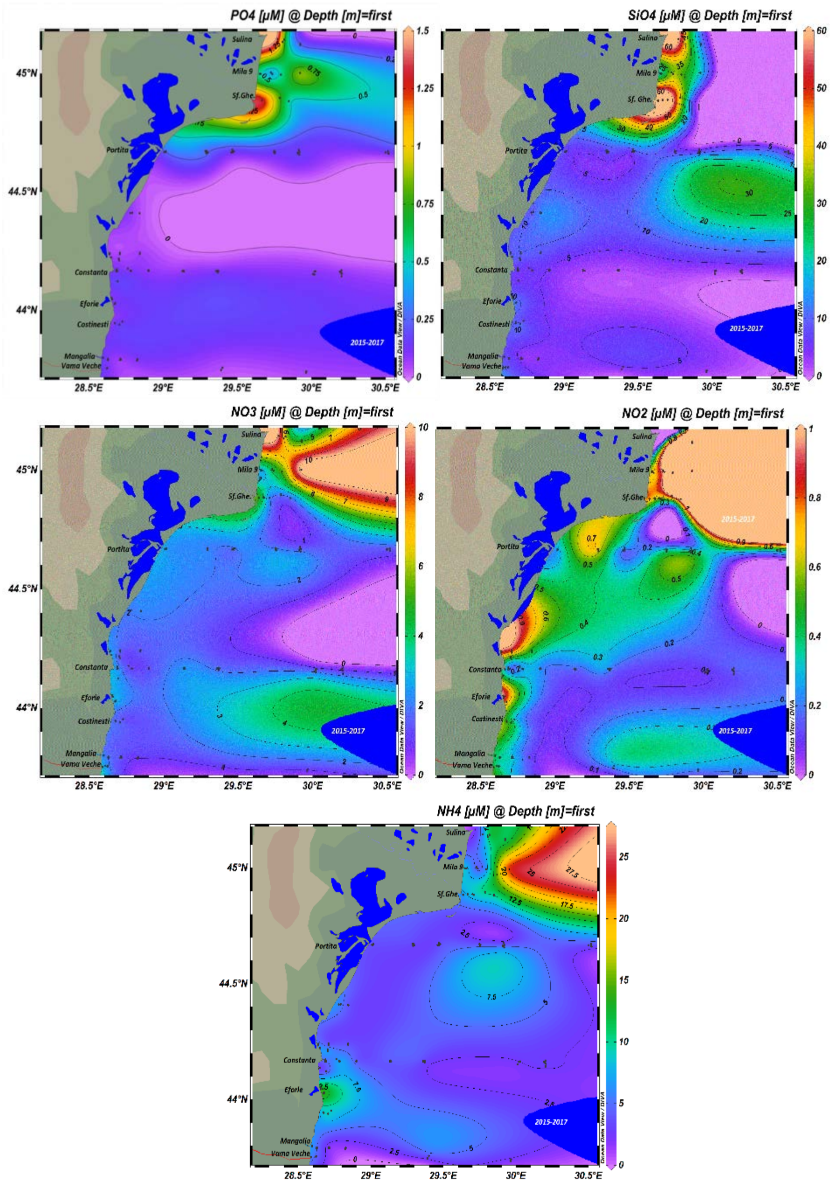

Fig. 2. Nutrients (PO_4-phosphate, SiO_4-silicate, NO_3-nitrate, NO_2-nitrite and NH_4-ammonium) surface distribution, Romanian Black Sea, 2015-2017.

In terms of nutrients, causes of eutrophication, we found a moderate risk to not achieve the Good Ecological Status (Descriptor 5) due to the exceeding of the proposed targets from the phosphate input of Danube's discharge and the coastal sources input of inorganic nitrogen (Table 2) (Boicenco et al., 2018).

Table 2. Nutrients environmental status (GES) - Romanian Black Sea, 2015-2017

Indicators	Transitional Waters 75th percentile	GES target	Coastal Waters 75th percentile	GES target	Marine Waters 75th percentile	GES target
DIP (as phosphate), µM	0.55	0.45	0.22	0.30	0.24	0.23
DIN (as sum of nitrate, nitrite and ammonium), µM	20.13	37.5	15.94	13.50	10.88	10.50

QUALITATIVE COMPOSITION

Phytoplankton

Analysing the taxonomic composition of phytoplankton in the Romanian Black Sea coastal waters, 199 species, varieties and forms of microalgae were identified, belonging to 8 taxonomic phyla with a minimum number of 105 species in the autumn, and a maximum one, of 171 species, in the summer.

Species of dinoflagellates were dominant in all samples; their number oscillated within quite tight limits, namely, the largest number of species was observed in the summer months - 65, followed by the spring months, with 49 species, and 34 species, in the autumn (Fig. 3).

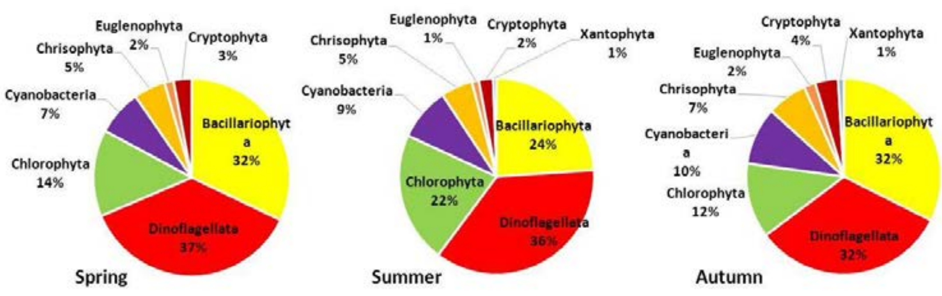

Fig. 3. Phytoplankton taxonomic composition in the Romanian Black Sea waters, 2015-2017.

Nine genera of dinoflagellates were observed in the communities: *Protoperidinium*, *Gonyaulax*, *Dinophysis*, *Amphidinium*, *Prorocentrum*, *Gyrodinium*, C*eratium*, *Heterocapsa*, the first two genera presented the higher specific diversity, each one with an approximately equal number of species, 11 and 10 respectively. The rest of the genera contributed up to four species - *Amphidinium*, *Prorocentrum* and *Gyrodinium*.

The second important group was diatoms, with the total number of species rising to 43, during the spring, and 41 in the summer, and their number was slightly lower in the autumn, 34 species, but equally with those of dinoflagellates.

Out of the 59 species of diatoms, most belong to the genus *Chaetoceros*, which contributed to the composition of communities with 17 species (*Chaetoceros curvisetus*, *Ch. socialis, Ch. similis*, *Ch. danicus*, *Ch. affinis, Ch. lorenzianus*, *Ch. muelleri*), followed, by the genus *Thallassiosira* (8 species), the most common being *T. parva*, *T. anguste-lineata*, *T. rotula*. Other representative genera for this group were *Cyclotella*, *Navicula*, *Nitzschia*/*Pseudo-nitzschia*, *Pseudosolenia*, *Melosira*, *Leptocylindrus*, *Skeletonema*.

Other groups that were better represented in the communities identified in all three seasons were the chlorophytes - 39 species, followed by a far lower number of cyanobacteria - 18 species. Of the chlorophytes, the richest ones were the genera *Tetraedron*, *Scenedesmus* and *Monoraphidium*, each represented by six species. Among the cyanobacteria worth mentioning is only the genus *Merismopedia*, with four species.

Microzooplankton

Analysing the taxonomic composition of tintinnids community in the Romanian Black Sea coastal waters, in the period 2016-2017, 25 species were identified, belonging to 10 genera with a maximum number of 17 species in the summer, and a minimum of 7, in the autumn.

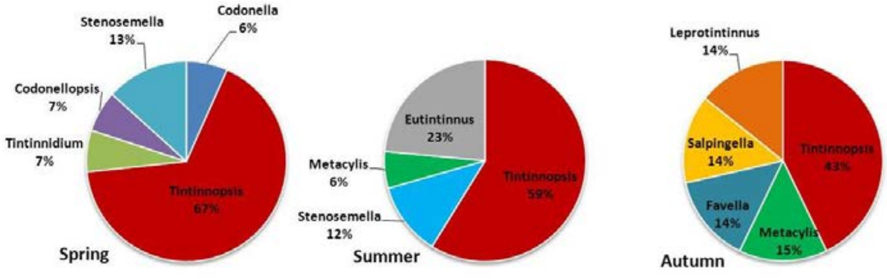

Fig. 4. Taxonomic composition of microzooplankton in the Romanian Black Sea waters, 2016-2017.

Seasonal distribution also shows that each season is characterized by different genera, except for the *Tintinnopsis* genus that has species present in all seasons with 10 species in the spring and summer and 3 species in the autumn respectively. This distribution is due to the ecology of the species and the environmental conditions specific to each season (Fig. 4).

In addition to the 1960-2000 period (Petranu, 1997, Moldoveanu & Timofte, 2004), 12 species were identified in the microzooplankton component, which study was recently restarted at the Romanian seaside. *Tintinnopsis baltica*, *T. compressa*, *T. karajacensis*, *T. urnula*, *T. mucicola* are mentioned in the literature as species common to the Black Sea basin (Gavrilova, 2005). *Eutintinnus apertus*, *E. lasus-undae*, *E. tubulosus*, *Salpingella decurtata*, *Tintinnopsis tocantinensis*, are species mentioned in the literature as invasive, who entered and acclimated in the Black Sea, during the last two decades (Gavrilova, 2005, Selifonova, 2012). *Eutintinnus pectinis*, another non-indigenous species reported as new in the Azov Sea in 2011 (Kreneva, 2013), was identified in the samples collected in the summer 2016, being the first recorded in the Black Sea basin (unpublished data). *Codonellopsis schabi*, was identified as a single isolated specimen in the marine waters of the Mangalia profile in the spring of 2017 and is not mentioned in the literature as present in the Black Sea Basin (Gavrilova & Dovgal, 2016, Balkis & Koray, 2014). It should be clarified if in the future *C. schabi* will develop populations or is only a „casual visitor” most probably introduced with ballast water.

Mesozooplankton

The specific diversity of the zooplankton varied according to the season, the maximum number of species (23) being registered in the summer and the minimum (15) in the spring.

It is remarkable the numerical dominance of the copepod species, in all seasons, the maximum number being reached in the summer and autumn, when species *Acartia clausi*, *Pseudocalanus elongatus*, *Calanus euxinus*, *Oithona similis*, *Paracalanus parvus* were the best represented, in the cold season, and *Centropages ponticus* in the warm season (Fig. 5).

The calanoid copepod *Oithona davisae* was for the first time signalled in the Sevastopol Bay of the Black Sea, in 2001, when only two isolated specimens were found in the samples (Zagorodnyaya, 2002). Subsequently, in 2005 and 2006, the species was again identified in the same location, but in much more abundant populations, represented by all stages of copepodites, including adults (Gubanovab & Altukhov, 2007). In the Romanian Black Sea region, *Oithona davisae* was registered from August 2010 (Timofte & Tabarcea, 2012) until 2014. In the next years, 2015 and 2016, the species was missing from the samples, to be again identified in the autumn of 2017, when it reached the highest density and biomass values in the waters in front of the Danube mouths.

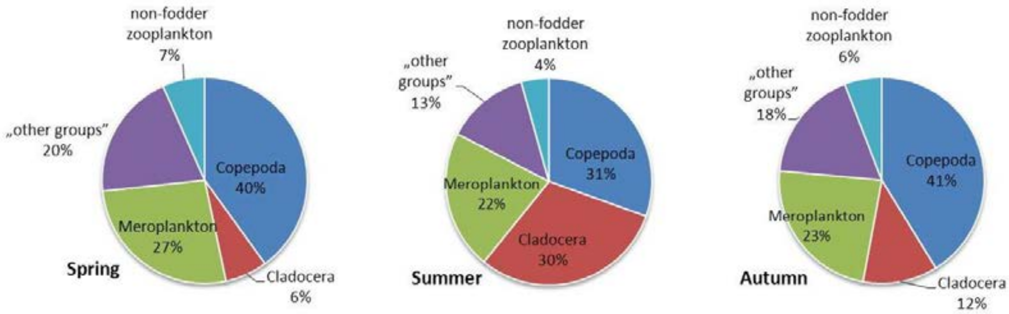

Fig. 5. Taxonomic composition of mesozooplankton in the Romanian Black Sea waters, 2016-2017.

The systematic group of *Cladocera* presented variations, with a maximum number of species in the summer season, and significant decrease in spring and autumn. This is due to environmental conditions, especially temperature, which plays an important role in the development of these organisms, high temperatures favouring their occurrence and development. The high number of Cladocera species is also due to the presence of the species pertaining to the genus *Bosmina*, *Chydorus* and *Daphnia* in the summer months, species occurring in the waters found in front of the Danube mouths.

Meroplankton and the organisms included in the generic category "other groups" were less represented, the maximum number of meroplanktonic forms being reached in the summer, the „other groups" registering a constant number of species in all seasons.

The non-fodder zooplankton, whose sole representative is the *Noctiluca scintillans* dinoflagellate, was present in all seasons.

Gelatinous zooplankton

Analysing the samples, three species were identified in the spring and summer of 2017: the scyphozoa *Aurelia aurita*, and the ctenophores *Pleurobrachia pileus* and *Mnemiopsis leidyi*.

The ctenophore *Beroe ovata* was not identified in any of the analysed samples, because they were collected before its maximum development season (autumn), the species having a peak in September, according to several bibliographic sources (Shiganova, 2001).

QUANTITATIVE ANALYSIS

Phytoplankton

Regarding the distribution of phytoplankton densities and biomasses on water bodies and seasons, it was noted that the values ranged between 840 and $3.14 \cdot 10^6$ cells/L, 1 and 3035 mg/m^3 respectively. The minimum values were recorded during spring, in marine waters. The maximum values of density were recorded during autumn, in transitional waters and the maximum values of biomass were recorded during summer in coastal waters (Fig. 6).

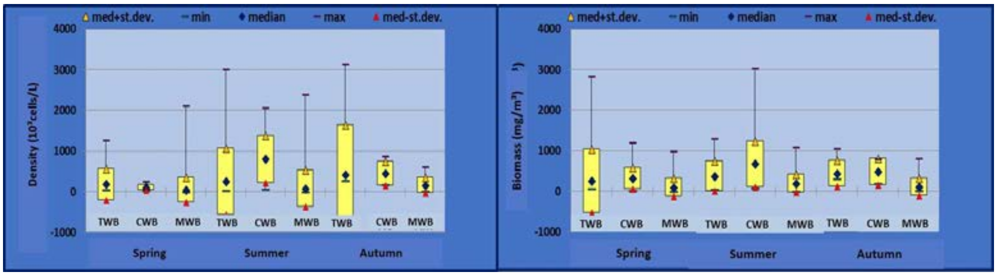

Fig. 6. Variations in the abundance and biomass of phytoplankton by water bodies and seasons, 2015-2017.

The average values ranged between $103 \cdot 10^3$ cells/L and $1.34 \cdot 10^6$ cells/L, 190 and 922 mg/m^3 respectively. The minimum average values were recorded during spring and autumn in coastal, respectively, marine waters and the maximum ones in autumn.

Regarding the dynamics of the main taxonomic groups of phytoplankton according to the average density recorded in the period 2015-2017, the diatoms dominated the phytoplankton communities during summer and autumn reaching 80% and, 76%, respectively, of the total average density in coastal waters.

During autumn, in the transitional waters, the species belonging to other groups contributed up to 74% of the total average density, mainly due to the development of the cyanobacteria *Planktolyngbya circumcreta* ($2.21 \cdot 10^6$ cells/L, Portiţa 1).

In average biomass, dinoflagellates were dominant in all water bodies, being present in higher proportions in spring (76-90%), compared to autumn (47-59%) and summer (43-50%). The higher values recorded in spring were due to the development of the dinoflagellate *Protoperidium granii*, especially in transitional and coastal waters.

Microzooplankton

In terms of the quantitative structure of the tintinnid community, significant seasonal variations in water bodies were revealed (Fig. 7).

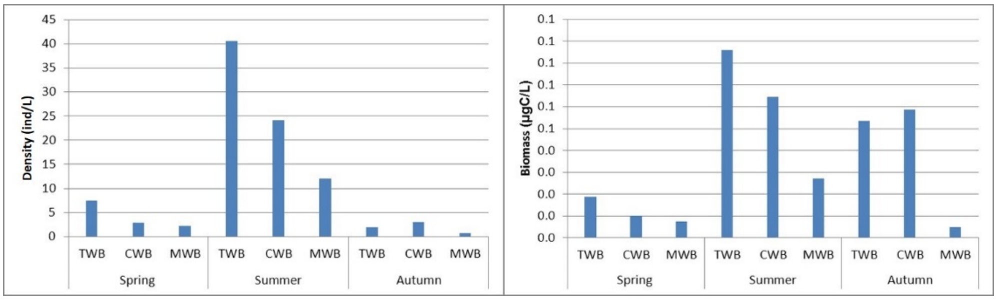

Fig. 7. Variations of microzooplankton abundance and biomass by water bodies and seasons, 2016-2017.

In the transitional waters the average densities and biomasses ranged between 2 - 41 ind/L and 0.02 - 0.09 µgC/L respectively with the maximum values in summer. The minimum density values were recorded during autumn, while the lowest biomass values were recorded during spring. Regarding the dominant species of tintinnid, in this water body, *Tintinnopsis parvula* has recorded the highest average density value (53%) in spring, while *Tintinnopsis minuta* dominated in abundance (63%) in summer and *Favella ehrenbergii* (64%) in autumn, respectively.

In coastal waters, average densities and biomass were lower than in transitional water in the spring and summer, but not in the autumn. The average densities and biomasses ranged among 3 - 24 ind/L and 0.01 - 0.06 µgC/L respectively, with the maximum value in summer. The dominant species in the abundance for each season were *T. parvula* (34%) in spring, *Eutintinnus pectinis* (55%) in summer and *Favella ehrenbergii* (48%) in autumn.

Marine waters were characterized by the lowest values of average density and biomass, 1 - 12 ind/L and 0.005 - 0.03 µgC/L respectively with the maximum values in summer. For this type of water, the dominant species in the abundance were *Tintinnopsis karajacensis* (29%) in spring, *Tintinnopsis minuta* (47%) in summer and *Salpingella decurtata* (49%) in autumn.

Mesozooplankton

During the study, the total zooplankton exhibited variations in density and biomass, reaching a maximum (34,930 ind/m^3, 2,547 mg/m^3) in the autumn in the transitional waters, then it recorded minimum values (a density of 7,222 ind/m^3 and a biomass of 180 mg/m^3) in the spring in coastal waters (Fig. 7).

From the quantitative point of view, the summer and autumn were dominated by the non-fodder zooplankton. Thus, in the transitional and coastal waters, dinoflagellate *Noctiluca scintillans* reached maximum densities and biomasses (27,945 ind/m^3, 2,459 mg/m^3) in the autumn. In the spring, the fodder zooplankton dominated, the maximum values being recorded in the coastal waters (Fig. 8).

As for the structure of the fodder zooplankton in the spring season of the three years of observations, we have to remark the dominance of the copepods in all water bodies, with the maximum density and biomass found in the coastal waters, 4,950 ind/m^3 and 93 mg/m^3 respectively).

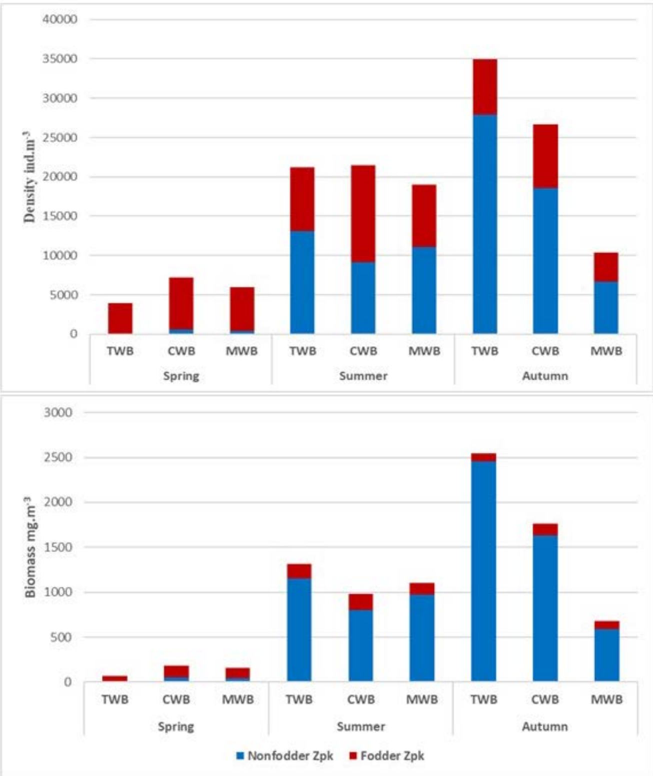

Fig. 8. Quantitative structure of mesozooplankton by water bodies and seasons, 2015-2017.

Unlike the spring, we must note the dominance of the meroplanktonic forms, both in the summer and autumn, which reached its maximum development in coastal waters, in both seasons. The Cladocera group was best represented in summer, reaching their highest values in coastal waters. Species pertaining to the generic category "other groups" were poorly represented in terms of quantity.

Gelatinous zooplankton

In 2017, in all bodies of water, in the spring season, there were high densities of the scyphozoa *Aurelia aurita*. In transitional waters, from the Portita area to the Danube mouths, where the depths reach 20 meters, *A. aurita* recorded its highest values, with a density of 6.5 ind/m^3; in the coastal waters also the scyphozoa *Aurelia Aurita* dominated with a density of 1.17g/m^3, followed by the *Pleurobrachia pileus* ctenophore, with a density of 0.66 ind/m^3 in marine waters (Fig. 8).

In summer, in the transitional waters, the highest values of the density were recorded by *A. aurita* - 23 ind/m^3, followed by the ctenophore *Mnemiopsis leidyi*, with a density of 15.24 ind/m^3. Both species occurred also in the coastal and marine waters but with lower values, *P. pileus* being the dominant species, with numerical values of 6.2 ind/m^3 in coastal waters, and 6.8 ind/m^3 in marine waters (Fig. 9).

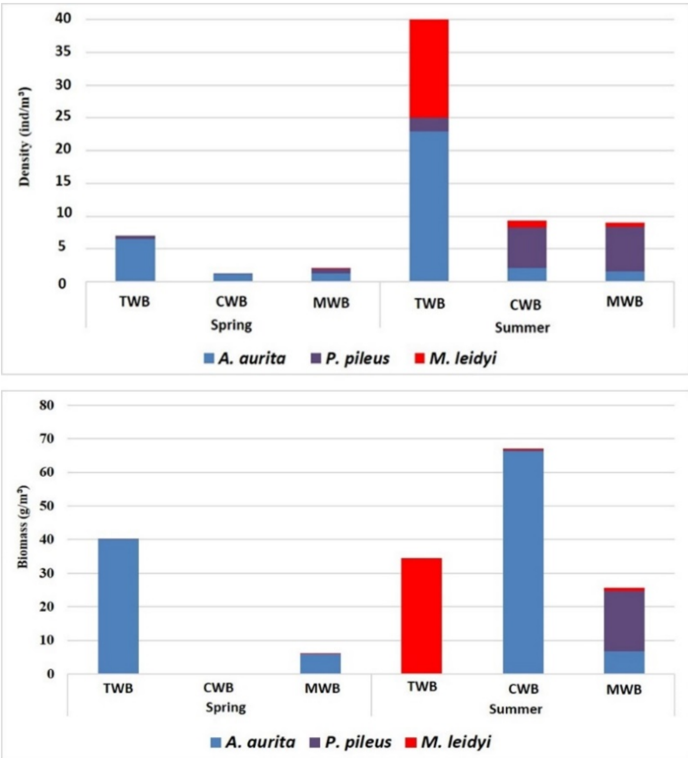

Fig. 9. Quantitative structure of gelatinous zooplankton by water bodies and seasons, 2017.

In spring, in the transitional waters, *A. aurita* registered the highest values, with a biomass of 40.1 g/m^3; in coastal waters, the biomass values of the gelatinous zooplankton were very low, and in the marine waters *A. aurita* dominated, with a biomass of 5.9 g/m^3.

In the transitional waters, during the summer, *M. leidyi* recorded the highest biomasses, with 34.23 g/m^3; in the coastal waters, *A. aurita* dominated the community, with 66.25 g/m^3, and in the marine waters the *P. pileus* ctenophore was dominant, with biomasses of 18 g/m^3 (Fig. 9).

EVALUATION OF THE ECOLOGICAL STATUS OF THE MARINE ENVIRONMENT

During the cold and warm seasons of the period 2015-2017, all the three bodies of water from the Romanian littoral attained "good ecological status", both based on the phytoplankton and mesozooplankton metrics Regarding the coastal waters, the value of phytoplankton biomass (1.204 mg/m^3) exceeded the target value (950 mg/m^3), defining this water body as "non-GES" (Table 3).

Table 3. Assessment of the ecological status of water bodies based on the phytoplankton biomass quality element (mg/m^3), during summer

Type of water body	Target value (mg/m3)	Recorded value 2015 -2017 (90 Percentile)	Ecological status
Transitional waters			
Sulina - Periboina	3000	1139	GES
Coastal waters			
Periboina – Vama Veche	950	1204	Non-GES
Marine waters			
Sulina Vama Veche	800	566	GES

For the mesozooplankton component, in cold season, all three water bodies recorded biomass values for good ecological status. In warm season, the good ecological status was not attained, all three water bodies being defined as "non-GES" by the values obtained using percentile 50 (Table 4).

As compared to the 1960-2000 period (Petranu, 1997; Moldoveanu & Timofte, 2004), we are able to identify major changes in the diversity and distribution of the tintinnid community meaning that a number of new species for the Romanian Black Sea coast have been identified and also some old ones have not been found (e.g. *Helicostomella subulata*, *Tintinnopsis lobiancoi*). Among the newly identified species there are also six invasive species, that is why the analysis of this component responds to the D2 Descriptor. ***It should be noted that all this new information about tintinnids community comes after almost two decades, where analysis was neglected.***

Table 4. Evaluation of mesozooplankton's ecological status indicators, based on the biomass values (mg/m^3)

Type of water body	Mesozooplankton		Copepoda		*Noctiluca scintillans*	
	Target value	Recorded value	Target value	Recorded value	Target value	Recorded value
	Cold season (November – April)					
TWB	>15	30	> 10	24	< 70	3
CWB	> 30	89	> 15	23	< 100	46
MWB	> 15	83	> 13	78	< 60	51
	Warm season (May - October)					
TWB	> 240	48	> 45	14	< 240	988
CWB	> 210	120	> 65	6	< 350	1249
MWB	> 70	43	> 45	31	< 60	209

Even the biomasses of *Mnemiopsis leidyi* ctenophore has reached GES in the cold season in all three water bodies, but in the hot season in coastal and marine waters, GES was also reached, yet in the transitional waters it recorded Non-GES (Table 5).

Table 5. Evaluation of the ecological status based on *Mnemiopsis leidyi* biomass indicator

Type of water body	Spring 2017		Summer 2017	
	Target value	Recorded value	Target value	Recorded value
Transitional waters	≤ 4 g/m3	GES	≤ 4 g/m3	Non-GES
Coastal waters		GES		GES
Marine waters		GES		GES

DISCUSSION

Prior work, the importance of plankton for the Black Sea's ecosystem status was duly recognised. Recent assessments of the changing environmental conditions of the Black Sea evidence that the north-western shelf (NWS) had improved to a certain extend in the last decades (e.g. in 2000-2010) due to decreasing nutrient loads from the rivers (Oguz & Velikova, 2010) but suffered a regime shift defining a quasi-stable state with interannual fluctuations (Oguz & Velikova, 2010; Daskalov et al., 2017). We found that in Romanian waters in summer the Good Ecological Status (GES) was not achieved for most of the water bodies and criteria meaning that pelagic habitats are not able to provide ecosystem services, particularly food provision for supporting food webs. Romanian Black Sea waters were dominated in summer by *Noctiluca scintillans* (in as many as 86% of stations) indicating a degraded ecosystem in the form of an intermediate production state (Oguz & Velikova, 2010) between healthy and eutrophic state. Additionally, *Noctiluca* might have a shifting role in planktonic food web, from mainly exerting top-down control during most of its pelagic life to fuelling bottom-up processes due to the liberation of intracellular nutrients during senescence (Zhang et al., 2017).

The magnitude of *Noctiluca scintillans* summer proliferation exceeded the GES thresholds from 3.5 times (in coastal and marine waters) to 4.1 times in waters with lower salinity (transitional waters). Overall, the normal range data (N=96) showed that the population dynamics of *Noctiluca scintillans* was mainly governed by temperature (r =0.55), dinoflagellate densities (r=0.47), salinity (r=-0.44) and diatoms biomass (r=0.39) without significant direct correlations with nutrients (Fig.10 and Fig.11). Throughout the entire study nutrients availability was phosphorus limited with few exceptions (N=5, representing 2.5% from the total number of samples) in spring and autumn 2016-2017 when N/P oscillated within 10.3-14.8 because of increased phosphate concentrations. This limitation might represent an advantage to dinoflagellates proliferation which were dominant in average biomass due to their swimming ability, enabling them to migrate vertically to exploit nutrient gradients (Smayda & Reynolds, 2001).

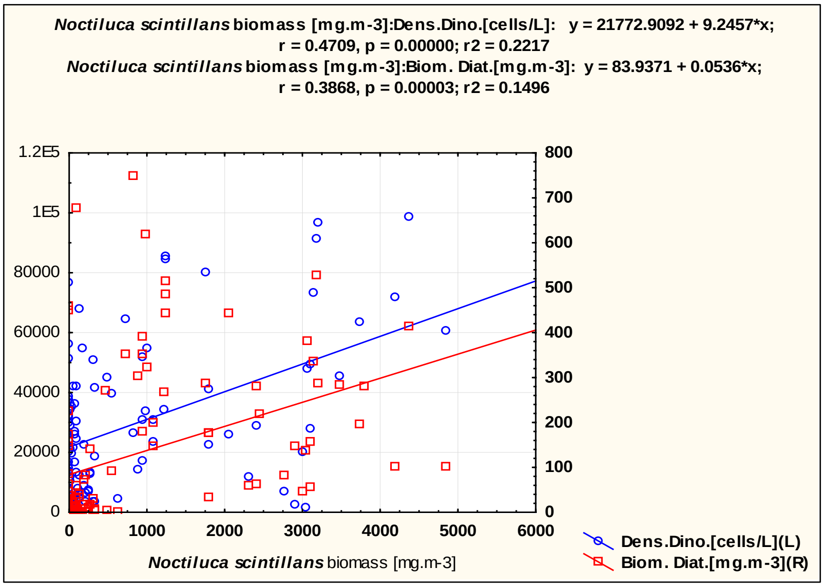

Fig. 10. Scatterplot of temperature (°C) and salinity (PSU) against *Noctiluca scintillans* biomass (mg/m^3).

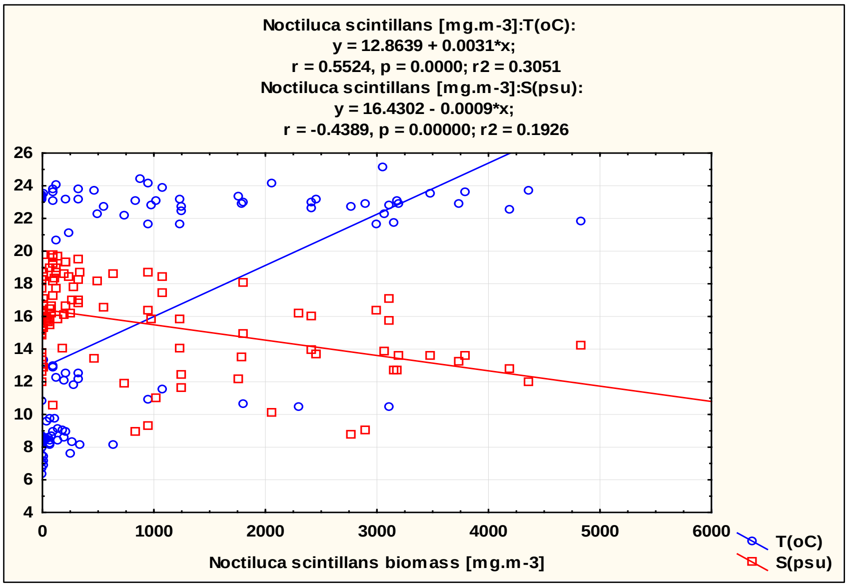

Fig. 11. Scatterplot of *Noctiluca scintillans* against dinoflagellates density (cells/L) and diatoms biomass (mg/m^3).

Other studies found that phytoplankton assemblages often are rich in diatoms at the onset of *N. scintillans* proliferations (Mohanty et al., 2007), and high numbers of *N.scintillans* have been observed concurrently with a high biomass of diatoms (Kiørboe & Titelman, 1998; Dela-Cruz et al., 2002; Turkoglu, 2013; Kopuz et al., 2014). In our study we observed higher *Noctiluca* abundance significantly associated with diatoms biomass in marine waters in summer (r = 0.57) and autumn (r = 0.64).

Moreover, along the Romanian littoral, the nutrients supply was pulsed, mainly because of the Danube's discharge, coastal sources and water circulation and contribute to higher phytoplankton biomasses because of a larger temporal mismatch between the growth of phytoplankton and of their zooplankton grazers (Svensen et al. 2002).

CONCLUSIONS

Generally, the significant influence on chlorophyll *a* concentration is exerted by salinity (r=-0.64), nitrate (r=0.55) and nitrite (r=0.53) levels. The seawater temperature significantly supports the dinoflagellates density development (r=0.53).

It was identified a moderate risk to not achieve the Good Ecological Status for nutrients due to the exceeding of the proposed targets. The main causes are the phosphate input of Danube's discharge and the coastal sources input of inorganic nitrogen.

During 2015-2017, 199 species of microalgae, belonging to 8 taxonomic groups, were identified in the Romanian Black Sea waters, the minimum number (105 species) being identified in the autumn season, and the maximum (171 species) in the summer.

The dinoflagellates dominated, representing 33% from the total number of species, followed by diatoms (30%) and chlorophytes and cyanobacteria (20% and 9% respectively). The rest of the algal groups (*Chrysophyta*, *Euglenophyta* and *Cryptophyta*) achieved only 8% of the qualitative composition.

Speaking about the distribution of phytoplankton densities and biomass by seasons, the highest average density was recorded in autumn ($1.34 \cdot 10^6$ cells/L), in transitional waters, and in the case of average biomass, in summer (821 mg/m^3) in coastal waters.

The dominance of diatoms in density (56-80%) was observed, except in the autumn, in transitional waters, when cyanobacteria dominated by 74% of the total. In biomass, dinoflagellates dominated the phytoplankton community in all studied seasons.

The value of phytoplankton biomass for the whole analysed period exceeded the target value, thus the coastal waters were in Non-GES status.

During 2016-2017 microzooplankton was present with 25 identified species, belonging to 10 genres with a maximum number of 17 species in the summer, and a minimum of 7, in the autumn. Among the species identified in the samples, 12 (6 invasive) are new to the inventory list of the Romanian Black Sea waters, underlying that the period during which the component has not been investigated is almost two decades.

Regarding the microzooplankton quantitative analysis during the analysed period, the density and biomass values were high in transitional waters, gradually decreasing in coastal and marine ones. The dominant species was *Tintinnopsis parvula* for transitional and coastal waters and *Salpingella decurtata* for marine waters.

During 2015-2017, the total number of mesozooplanktonic species was reached in the summer season. Regarding the qualitative structure, the copepods dominated in all three seasons, followed by Cladocera, with the maximum of species in the summer season. The copepod *Oithona davisae* was still present in the communities, but only in the samples collected in autumn 2017.

The non-fodder zooplankton dominated in quantitative terms both in the summer and autumn seasons, as opposed to the spring season, when the zooplankton communities were dominated by the fodder species.

Within the zooplanktonic trophic component, the group of copepods dominated the spring season, but in the summer and autumn the meroplankton component prevailed. Other Groups and Cladocera category were better represented, in terms of quantity, in the summer months, in the transitional waters; in coastal and marine waters it reached the status of "Non-GES". In the warm season, the Non-GES status was reached in all water bodies.

The maximum diversity of the gelatinous zooplankton was recorded in the summer season in the transitional waters.

In the spring season the scyphozoa *Aurelia aurita* dominated all the three bodies of water; in the summer season, in the transitional waters, *A. aurita* was the dominant species, followed by the ctenophore *Mnemiopsis leidyi*. In coastal and marine waters, the dominant species was the ctenophore *Pleurobrachia pileus*. The biomass of *Mnemiopsis leidyi* recorded Non-GES only in summer in the transitional waters.

For in-depth ecological status evaluation, continuous data acquisition is required in order to cover the study of seasonal dynamics of abiotic and biotic parameters. The data used in the present evaluation are less if we consider the spatial and temporal variations characteristic of the seas in the temperate zone. A reduced frequency of plankton sampling increases the risk of not surprising the temporal dynamics and differences in the collection frequency of each station may lead to a decrease in the confidence of the data used in future evaluations. Therefore, ecological status assessment of the pelagic habitats should be done in the future in a more integrated way, across indicators, criteria and descriptors to evaluate the progress towards GES.

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