CHARACTERIZATION OF ROMANIAN BLACK SEA PELAGIC HABITATS BASED ON MESOZOOPLANKTON DISTRIBUTIONAL PATTERN AND PROPOSAL OF A NEW INTEGRATIVE MESOZOOPLANKTON INDEX FOR ASSESSING THEIR QUALITY STATUS

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Abstract. The paper presents the mesozooplankton population characteristics and distributional patterns within the coastal, transitional, and marine waters of the Romanian Black Sea shelf in August 2021. The results point out the utility of zooplankton as a biological indicator for delimiting the pelagic habitats due to its features strongly linked with the environmental variables. The assessment of water bodies' quality based on the zooplankton indices agreed for the Black Sea according to the Water Framework Directive (2000/60/EC) and Marine Strategy Framework Directive (2008/56/EC), showed that in average 68% of indices were in GES, while the rest in non-GES. A new index, namely the Integrative Mesozooplankton Index (IMI), aiming to integrate the results of all three indices and percentage of water bodies in GES has been proposed. Three threshold classes for good, moderate and bad status have been established to delineate GES/nonGES threshold. At a threshold value of 6.6 – 9 proposed for the IMI GES, the overall quality status of the water bodies of the Romanian shelf in August 2021 did not achieve GES.

Key words: Romanian Black Sea, pelagic habitats, zooplankton, MSFD zooplankton-based indices, Integrative Mesozooplankton Index (IMI), quality status assessment

1. INTRODUCTION

The climate variability, anthropogenic stressors, and different mesoscale events may significantly influence the plankton composition and distribution, hence the key ecosystem services such as the water quality, productivity, and nutrients regenerations. Zooplankton phenological dynamic is also a good indicator of seasonal or interannual environmental changes as a result of timing covariation or mismatch between cycles of different trophic levels that is an important driver of changes in total population abundance and/or annual reproductive success. In spite of its capacity to retain during its life history the print of environmental changes, the zooplankton has rarely been used at the policy level for conservation and management of marine ecosystems services. To fill this gap, the development of efficient zooplankton indicators is needed to track progress against a suite of European Directives (Water Framework Directive, Marine Strategy Framework Directive, Common Fishery Policy, and Marine Spatial Planning) addressing water quality issues, securing food provision and regulatory services, and promoting ecosystem-based approach management. Moreover, zooplankton biomass and diversity were identified as one of the most mature Essential Ocean Variables (EOVs) of GOOS-BioEco (Miloslavich *et al.*, 2018).

Despite its relatively low diversity, the Black Sea zooplankton on the Romanian shelf contribute significantly to secondary production (Muresan *et al.*, 2020) and elicit a seasonal dynamic largely influenced by temperature, river inputs, spring /autumn blooms and decay, and predation (Vereshchaka *et.al*, 2019; Stefanova, 2015). The hydrodynamic and morphological features play also a significant role in zooplankton composition and distribution, and therefore it might be considered a suitable Biological Quality Element (BQE) to be used as a proxy indicator for the classification of waterbodies and tracking the hydrological changes (Ndah *et al.*, 2022).

The study analyses the composition and quantitative distribution of 2021 summer zooplankton (13-21 August 2021) in the Romanian pelagic habitats and the water quality status based on zooplankton populations' indicators. An integrative zooplankton index is proposed to assess the pelagic habitats' state.

2. MATERIAL AND METHOD

The zooplankton was collected in the period 13 -21 August 2021 (Annex - Table 1) in the waters of the Romanian shelf, covering all three water bodies (habitats) typologies (coastal, transitional, and marine) established according to the Water Framework Directive (WFD) (2000/60/EC) and Marine Strategy Framework Directive (MSFD) (2008/56/EC) transposed into the Romanian legislation (Boicenco *et al.*, 2018). The samples were taken along seven transects by vertical hauls from bottom to surface using a Juday plankton net with a 36 cm diameter opening and mesh size of 150 µm. In laboratory, a total of **23** samples (Fig. 1) were processed according to the methodology for zooplankton studies in the Black Sea (Alexandrov *et al.*, 2014).

2.1. Statistical analysis

Statistical analysis was performed in PRIMER v.7.0. ANOSIM was employed to show the significant statistical differences at p cut-off < 0.05) in zooplankton distribution within water bodies. The metric MDS Bray – Curtis similarity based on the bootstrap resampling and SIMPER analysis were conducted to show groups dissimilarities. All figures have been built with the help of free software ODV v.5.5.2 (Schlitzer, 2021) using DIVA algorithm.

2.2. Study area

The study area covers the entire Romanian shelf between 45.07° and 43.8° N latitude and 28.66° and 30.8° E longitude, from 15 to 149 m depth, comprising three water bodies (pelagic habitats) typologies. The northmost type, namely the transitional waters, is directly influenced by the freshwater input from Danube and lies between Sulina and Portita and eastward up to 10 – 15 m. The coastal waters are delineated between Portita and Mangalia and about 30 m eastward, whereas the marine waters cover the outer shelf beyond 30 m depth. Each unit is characterised by peculiar features as a result of Danube inputs, surface circulation and seasonal seawater stratification.

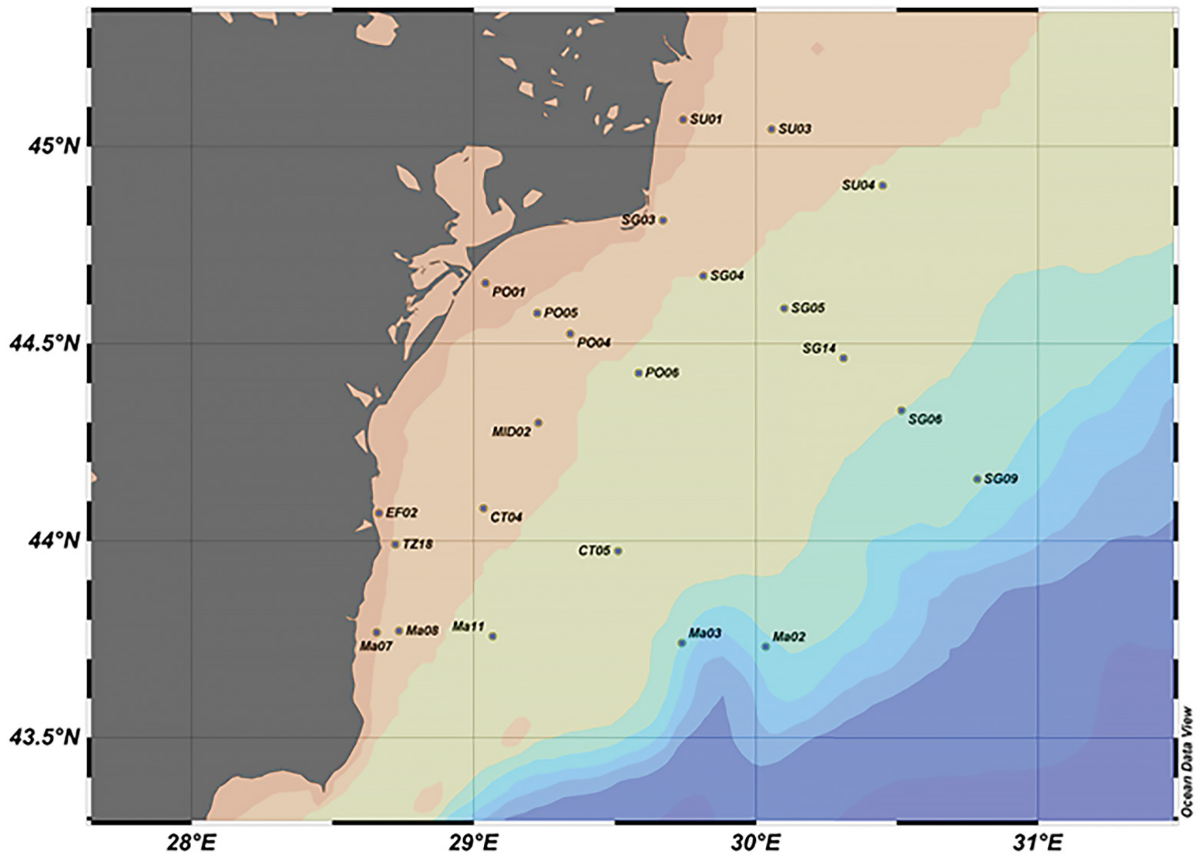

Fig. 1. The sampling map of zooplankton within the period 13 – 21 August on the Romanian Black Sea shelf.

The transitional waters support the most variable phisical-chemical parameters such as salinity, oxygen and nutrients input, depending on the river debits. Though the coastal waters could also experience similar conditions following the high freshwater flows periods, the hydrological features are much stable. The marine waters, beyond 40-50 m, are mostly influenced by the geostrophic forces connected with the global oceanic and atmospheric circulation. Due to Danube's buoyancy-driven inflows and the prevailing winds, the shelf is rich in nutrients, which boost biological production. The onshore and offshore meanders of the river's plume support the phytoplankton (Oguz *et al.*, 2004) and zooplankton production (Muresan *et al.*, 2020).

3. RESULTS

During the summer period (August 2021), the mesozooplankton populations' diversity structure was made of 18 taxa, represented by 65% of species belonging to holoplankton, 33%, and 2% to meroplankton (larvae of bivalves, gastropods, decapods, bryozoans, polychaetes, and phoronids) and ihtyoplankton (eggs and larvae of fish), respectively (Annex - Table 2).

The spatial variation of zooplankton species richness (16.1±1.51 taxa on average; CV%: 9.4) across the shelf mirrored the features associated with the relative vertical homogeneity of coastal/transitional waters, as opposed to thermic stratified marine waters due to seasonal thermocline. These differences were accounted for by the thermophilic cladocerans, copepods and meroplankton taxa (larvae of Mytilidae Rafinesque, 1815, *Anadara* Gray, 1847, Rissoidae Gray, 1847, Pyramidellidae Gray, 1840, *Upogebia pusilla* (Petagna, 1792), *Pisidia longicornis* (Linnaeus, 1767), *Athanas nitescens* (Leach, 1814), *Xantho poressa* (Olivi, 1792), *Pilumnus reticulatus* Stimpson, 1860, *Liocarcinus* Stimpson, 1871, *Palaemon* Weber, 1795, *Amphibalanus improvisus* (Darwin, 1854), in transitional and coastal waters (PO01, SU01, PO05, Ma07) and by the cryophilic copepods living under the thermocline (*Calanus euxinus* Hulsemann, 1991, *Pseudocalanus elongatus* (Brady, 1865)), in the marine waters, respectively. In turn, the zooplankton composition distribution within the surface layer of both coastal and marine waters was similar. The maximum taxa number (18) was found in marine stations on the Portiţa transect (PO05 and PO06) and two coastal southern stations (Ma07 and TZ18), while the minimum (11 taxa) within the marine station MID02. Copepods and cladocerans with 7 and 4 species, respectively formed the bulk of the holoplankton qualitative structure, whereas the decapods larvae (9 species), gastropods (Rissoidae Gray, 1847, Pyramidellidae Gray, 1840), bivalves and polychaetes (Spionidae Grube, 1850 and Syllidae Grube, 1850) that of meroplankton. The most frequently encountered ihtyoplankton larvae belonged to sprat (*Sprattus sprattus* (Linnaeus, 1758)), horse mackerel (*Trachurus trachurus* (Linnaeus, 1758)), mullet (*Mugil* (Linnaeus, 1758), *Sarda sarda* (Bloch, 1793), *Pomatomus saltatrix* (Linnaeus, 1766)) and turbot (*Scophthalmus maeoticus* (Pallas, 1814)).

Overall, more than 50% of the quantitative structure (abundance) of the zooplankton populations consisted of cladocerans, followed by copepods (about 22%) and meroplankton (11%). The thermophilic cladoceran *Penilia avirostris* Dana, 1849 ranked the first two places after density and biomass of total zooplankton population, with 1439.81 ind.m^{-3} and over 60 mg.m^{-3} respectively (Annex - Table 2). Overall, Cladocera made up to about 14% of the total zooplankton biomass, the greatest bulk of the populations being confined to the coastal waters (Fig. 2a). The copepods *Acartia (Acartiura) clausi Giesbrecht, 1889* and *Acartia (Acanthacartia) tonsa Dana, 1849*, on one hand, and *Centropages ponticus* Karavaev, 1895, on the other hand, amounted to 17% and 8% of total density and biomass respectively. Biomasses of *C. euxinus* Hulsemann, 1991 reached a peak at the deepest stations (149 and 118 m bottom depths) SG09 (2079 mg.m^{-3}) and MA02 (89.4 mg.m^{-3}) (Annex - Table 2), under the CIL layer. Meroplankton reached the maximum biomasses in the southern stations (Annex - Table 2 and Fig. 2c). Across the entire shelf, the highest total average biomass (146.73 mg.m^{-3}) was reached, in turn, by the prolific populations of chaetognath *Parasagitta setosa* (J. Müller, 1847), which represented about 34% of the total zooplankton biomass (Annex - Table 2).

The transitional and coastal waters showed no significant differences (ANOSIM statistic: 0.036, significance level %: 46.7) between them, whereas a significant statistical difference was noted between each of the above habitats and the marine one (ANOSIM statistic: 0.637, significance level %: 1.3 and ANOSIM statistic: 0.618, significance level %: 0.2, respectively). The R statistic between the first two habitats suggests differences rather within the groups than between the groups (Annex - Table 3).

The metric MDS Bray – Curtis similarity of trophic zooplankton densities based on the bootstrap resampling with replacement method (number of bootstraps per group: 100) figured the separation of communities within the three habitats (Fig. 3a). The distribution map of densities of trophic zooplankton in August 2021 shows good compliance with the statistical results. The southern coastal stations (*e.g.*, TZ18) stand out in terms of density reached due to the influence of the meroplankton community that added up to the total (Fig. 3b). The SIMPER analysis (Table 1) revealed an average dissimilarity of 51.04 between the groups 1 (transitional habitat) and 2 (coastal habitat) and a dissimilarity of 67.86 and 68.12 between the groups 1, 3 (marine habitat) and 2, 3, respectively.

ANOSIM test (Global R: 0.37; significance level of sample statistic: 0.4%) confirmed the differences between pelagic habitats in terms of biomasses of zooplankton. Hence, significant statistical differences were recorded between the transitional (group 1) and coastal waters (group 2), on one hand, and the marine waters (group 3), on the other hand (ANOSIM statistic: 1, 3 groups: 0.40, significance level %: 3.3; ANOSIM statistic: 2, 3 groups: 0.36, significance level %: 1.9), but no significant statistical difference was noted between the first two groups (ANOSIM statistic: 1, 2 groups: 0.39, significance level %: 13.3) (Annex - Table 4).

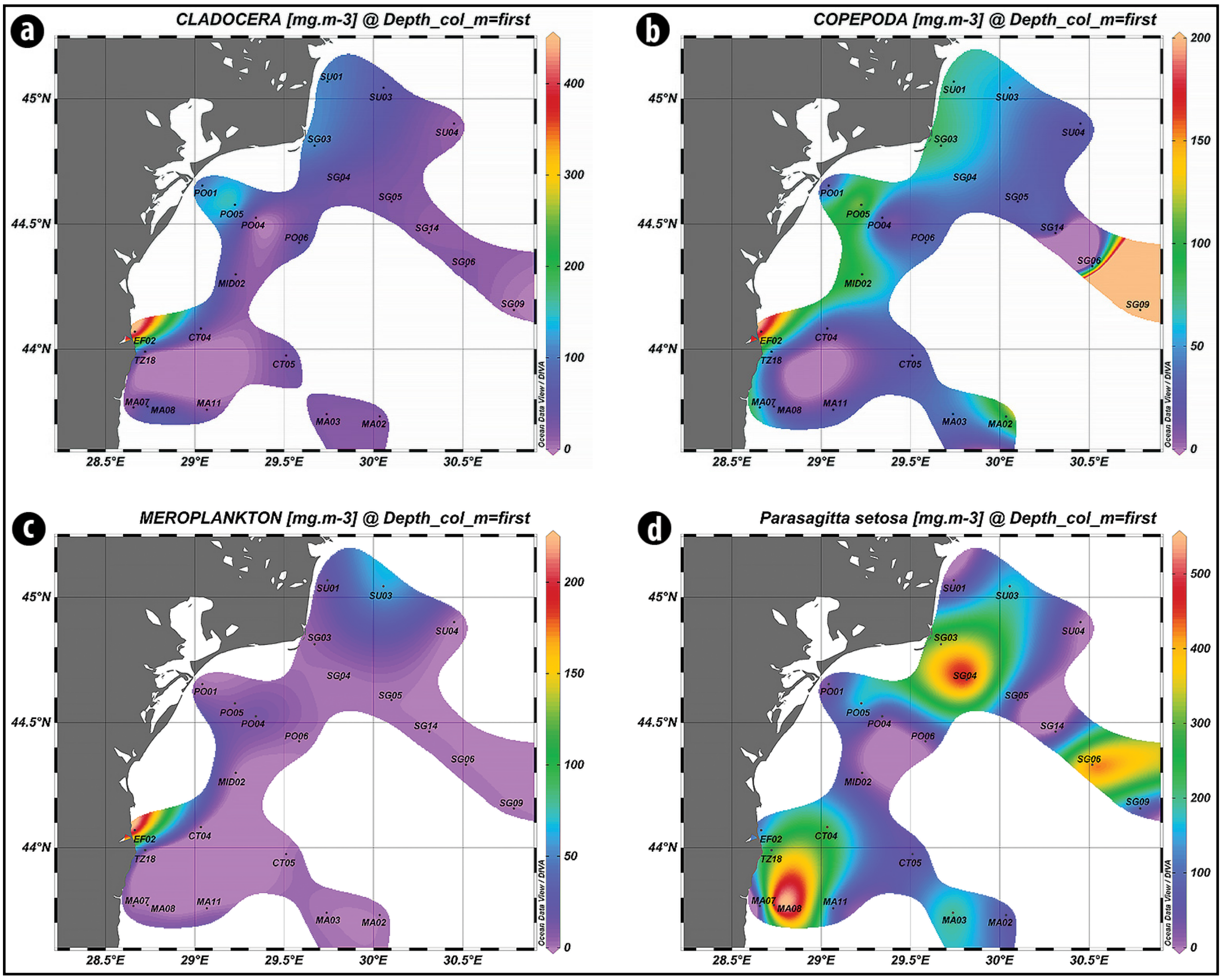

Fig. 2. The distribution of biomasses (mg.m-3) of cladocerans (**a**), copepods (**b**), meroplankton (**c**) and *P. setosa* (**d**) in the Romanian Black Sea waters (integrated on depth), in the period 13 -21 August 2021.

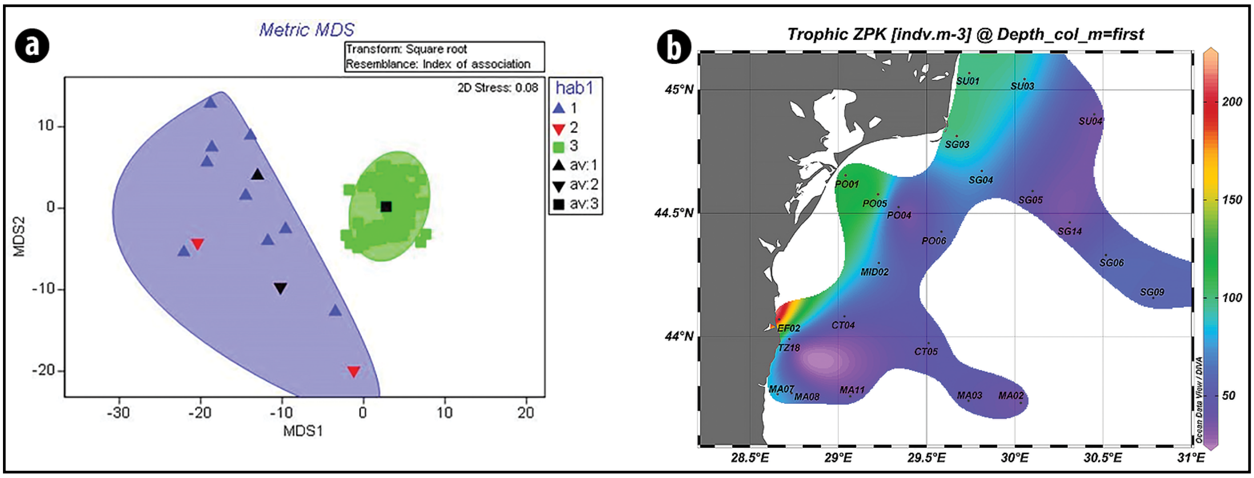

Fig. 3. (**a**) Multimetric dimensional analysis of similarity (average bootstrap resampling with replacement method) among the three water bodies/pelagic habitats (1, 2, 3) based on the Bray-Curtis index of similarity of squared root zooplankton densities (blue triangle **1**: transitional waters; **2**: inverse red triangle; coastal waters; green squares **3**: marine waters); (**b**) Distribution map of trophic zooplankton (squared root) densities (indv.m^{-3}) in the Romanian waters within 13 -21 August 2021.

Table 1. SIMPER test of dissimilarity between groups (habitats) and species abundance cumulative contribution (cut-off 70%).

Groups 1 & 2 **Average dissimilarity = 51.04**						
Species	**Group 1**	**Group 2**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum. %**
Evadne spinifera	4243.55	1948.28	11.89	0.98	23.23	23.23
Penilia avirostris	2146.15	4304.38	9.27	1.43	18.17	41.40
Noctiluca scintillans	0.00	1351.88	5.75	0.93	11.26	52.66
Pseudevadne tergestina	1593.06	2605.46	4.31	1.26	8.44	61.10
Pleopis polyphemoides	790.98	306.22	3.19	1.19	6.25	67.35
Centropages ponticus	841.35	1652.67	2.71	1.86	5.31	72.65
Groups 1 & 3 **Average dissimilarity = 67.86**						
Species	**Group 1**	**Group 3**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum. %**
Evadne spinifera	4243.55	466.13	22.98	1.25	33.87	33.87
Penilia avirostris	2146.15	787.40	9.83	1.95	14.49	48.36
Pseudevadne tergestina	1593.06	608.94	7.35	2.61	10.83	59.19
Pleopis polyphemoides	790.98	43.92	5.81	0.97	8.56	67.75
A. clausi + A. tonsa	1004.91	401.11	4.61	1.55	6.79	74.54
Groups 2 & 3 **Average dissimilarity = 68.12**						
Species	**Group 2**	**Group 3**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum. %**
Penilia avirostris	4304.38	787.40	13.35	1.53	19.59	19.59
Pseudevadne tergestina	2605.46	608.94	8.44	1.56	12.39	31.98
Noctiluca scintillans	1351.88	443.93	8.37	1.17	12.28	44.26
Centropages ponticus	1652.67	322.13	7.30	1.83	10.71	54.97
Evadne spinifera	1948.28	466.13	6.21	2.22	9.11	64.08
A. clausi + A. tonsa	1961.53	401.11	6.02	1.84	8.84	72.93

The metric MDS Bray – Curtis similarity of trophic zooplankton biomasses based on the bootstrap resampling with replacement method (number of bootstraps per group: 100) differentiated the communities over the three water bodies (Fig 4). The SIMPER test (Table 2) showed that *P. setosa* contributed significantly to delimiting the three water bodies. The highest zooplankton biomasses were reached in the southern sector as well as in a few of the offshore stations (Fig. 4b).

3.1. Pelagic habitats quality assessment

Three individual and one integrative zooplankton indices were used to evaluate the ecological status of the pelagic habitats (water bodies) during the study period (Table 3).

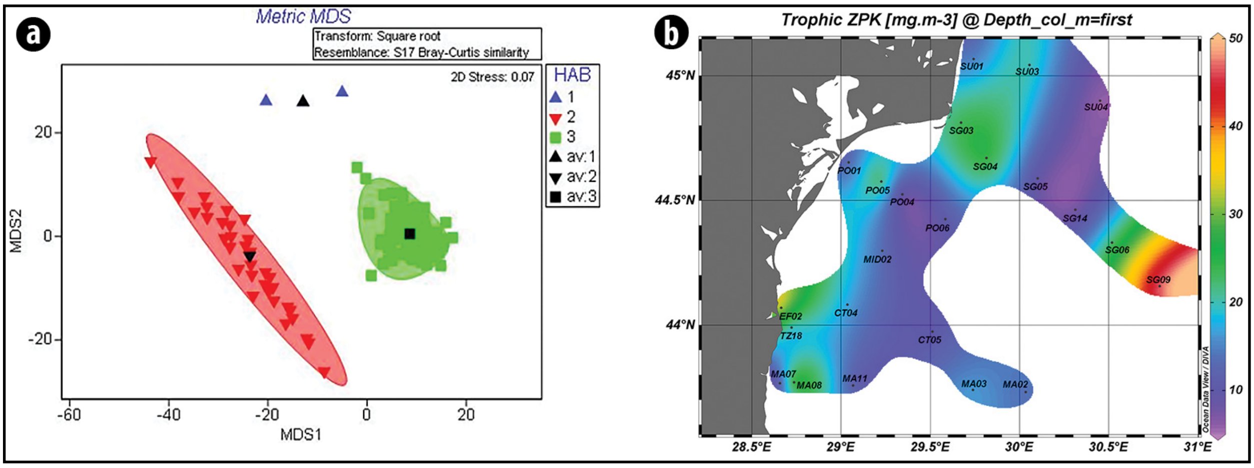

Fig. 4. (**a**) Multimetric dimensional analysis of similarity among the three water bodies/pelagic habitats (1, 2, 3) based on the Bray-Curtis index of similarity of squared root zooplankton biomasses (average bootstrap method); (**b**) Distribution map of trophic zooplankton (squared root) biomasses in the Romanian waters (mg.m^{-3}) within 13 -21 August 2021.

Table 2. The SIMPER test of dissimilarity between groups (habitats) and species biomass cumulative contribution (cut-off 70%).

Groups 1 & 2 **Average dissimilarity = 70.22**						
Species	**Group 1**	**Group 2**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
P.setosa	21.98	255.77	21.57	0.89	30.71	30.71
N.scintillans	0.00	118.97	16.89	0.91	24.05	54.77
P.avirostris	75.12	150.65	11.78	1.17	16.77	71.54
Groups 1 & 3 **Average dissimilarity = 70.25**						
Species	**Group 1**	**Group 3**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
P. setosa	21.98	164.98	22.75	1.14	32.38	32.38
Penilia avirostris	75.12	27.56	12.27	1.66	17.47	49.86
N.scintillans	0.00	39.07	7.90	1.47	11.25	61.10
Centropages ponticus	30.32	11.45	5.08	1.44	7.23	68.33
Evadne spinifera	16.97	1.86	3.85	0.99	5.48	73.81
Groups 2 & 3 **Average dissimilarity = 69.36**						
Species	**Group 2**	**Group 3**				
	Av.Abund	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
P. setosa	255.77	164.98	22.05	1.12	31.79	31.79
N.scintillans	118.97	39.07	14.46	1.06	20.85	52.64
P.avirostris	150.65	27.56	11.31	1.03	16.30	68.94
Centropages ponticus	65.87	11.45	5.27	2.77	7.60	76.55

Table 3. The Ecological State of the transitional (T), coastal (C), and marine waters (M) of the Romanian shelf based on the indices *Biomass of Copepods, Trophic Mesozooplankton Biomass* and *Biomass of Noctiluca scintillans* indices in August 2021 (red – non GES; green - GES)

Station	Water body (Pelagic habitat)	GES	Copepoda (mg.m-3)	GES	Mesozooplancton (mg.m-3)	GES	*Noctiluca scintillans* (mg.m-3)	%of stations in GES
P001	T	> 45	[red]	>240	[red]	240	[green]	0.5
SU01	T		[green]		[red]		[green]	
TZ18	C	> 65	[green]	>210	[green]	350	[green]	0.83
Ma07	C		[red]		[green]		[green]	
EF02	C		[green]		[red]		[green]	
P005	C		[green]		[green]		[green]	
SG03	M	> 45	[green]	>70	[green]	60	[red]	0.7
CT05	M		[red]		[green]		[green]	
Ma03	M		[red]		[green]		[green]	
SG05	M		[red]		[green]		[green]	
MID02	M		[green]		[green]		[green]	
CT04	M		[red]		[green]		[green]	
SG14	M		[red]		[green]		[green]	
SU04	M		[red]		[red]		[green]	
Ma02	M		[green]		[red]		[green]	
Ma11	M		[red]		[green]		[green]	
Ma08	M		[red]		[green]		[green]	
P004	M		[red]		[green]		[red]	
P006	M		[red]		[green]		[green]	
SU03	M		[green]		[green]		[green]	
SG04	M		[green]		[green]		[red]	
SG06	M		[red]		[green]		[green]	
SG09	M		[green]		[green]		[green]	
			0.43		0.78		0.87	**4.24**

The three indices are:

1. *The Biomass of copepods*;
2. *The Trophic Mesozooplankton Biomass*, and
3. The *Biomass of Noctiluca scintillans.*

The values of our study were compared with the historical references (the average values of 1960 – 1969 for the Good Ecological Status (GES) and those of the period 1977 – 2002 for the Bad Ecological State). A new tool consisting of an integrative index, namely the Integrative mesozooplankton Index (IMI), was proposed to condense the information of all three individual indices.

1. *The Biomass of Copepods (BM)* exceeded the minimum threshold of >45 mg.m^{-3} in 50% of the transitional stations and in 35.29% of the marine ones, whereas the threshold of >65 mg.m^{-3} set for the coastal waters has been achieved in 75 % of the stations. In total, according to the evaluation performed based on this index indices, GES has been reached in 43.47% of the stations (Table 3).

2. *The Trophic Mesozooplankton Biomass (MZB)* failed to reach the GES threshold of >240 mg.m^{-3} in all transitional stations. In 75% of the stations of coastal waters, the GES threshold of >210 mg.m^{-3} has been achieved, while the threshold of >70 mg.m^{-3} has been achieved in 88.23% of the marine stations (Table 3). In total, GES has been achieved in 78.26% of stations.

3. *The Biomass of Noctiluca scintillans* (BNS) did not exceed the GES threshold of 240 mg.m^{-3} and of 350 mg.m^{-3} in either of the transitional or coastal stations, while GES has been achieved in 82.35% of the marine waters, where the threshold of 60 mg.m^{-3} was exceeded in only 3 out of 17 stations. In total, the GES has been achieved in 87% of the stations (Table 3).

The Integrative mesozooplankton Index (IMI) was calculated as a weighted product of sum of percentages of stations in GES in each habitat and the sum of percentages of stations in GES according to each indices (Eq 1). A maximum value of 9 (attributing a value of 1 for each element of the matrix) was proposed for IMI, while the thresholds for GES/ non GES have been obtained by considering the 90, 75 and 25 percentages, respectively of the maximum IMI value (Table 4).

$$IMI = \sum (\%S_{tr}, \%S_{co}, \%S_m)^* \sum(\%S_{cop}, \%S_{MZ}, \%S_{NS}) \quad \text{(Eq. 1)}$$

where:

$\%S_{tr}$, S_{co}, S_m – % of transitional, coastal and marine waters stations in GES;

$\%S_{cop,}$ $_{\%}S_{MZ,}$ $_{\%}S_{NS}$ – % of stations in GES according to the BM, MZB, BNS.

Table 4. The EQS thresholds of GES for the IMI

Thresholds	IMI	GES
90th	8 <8.1 <9	GOOD
75th	6< 6.75< 8	MODERATE
25th	< 2.25< 6	BAD

Following the formula given in **Eq. 1**, the IMI was 4.24, we concluded that the Romanian waters in August 2021 did not achieve GES.

4. DISCUSSIONS AND CONCLUSION

The zooplankton abundances in the Romanian waters of the Black Sea shelf in August 2021 ranged between 1314.5 and 40,357.7± 8589.78 ind.m^{-3} as density and between 37.36 and 2171.16±459.52 mg.m^{-3} as biomass, with large spatial variations among the three water bodies. The coastal and transitional waters as opposed to marine ones featured significant higher zooplankton abundances as a result of different forcing mechanisms such as Danube's proximity, thermic regime and ageostrophic water flow, acting most of the time as predicting variables. Therefore, zooplankton is generally deemed a suitable Biological Quality Element (BQE) to be used as a proxy indicator for the classification of waterbodies and recording the hydro-climatic changes (Ndah *et al.*, 2022).

Our study brings arguments in favour of using zooplankton in future studies for defining ecoregions (Olson *et. al.*, 2002) and particular pelagic habitats of the Black Sea. The 2022 EUNIS (Eionet, 2022) classification system of the Black Sea contains a series of pelagic habitats (51) that often have been inadequately adapted from the Mediterranean and/or Baltic habitats. In contrast to the benthic habitats which have lately received increasing attention, in the case of pelagic habitats further research is needed in order to improve their classification at the national and regional levels. From the management point of view, the ecosystem-based approach would highly benefit from including the complex habitats such as the benthic-pelagic ones (merely defined in the EUNIS – code X30) into the classification system.

Global efforts for bio regionalization of the marine realm (Briggs, 1974, 1995; Longhurst, 1998; Kelleher *et al.,* 1995) were recognized for their utility in the management of fisheries, pollution, habitat restoration, productivity, socioeconomics, and governance (Spalding *et al.*, 2007). In the scope of fishery management, ICES defined, for example, the IOS Zooplankton Regions. Recently, the importance of ecoregions in the Black Sea was highlighted by Boero *et al.*, 2016 and Öztürk *et al.*, 2017, who studied the connectivity of MPAs by the means of dispersal of propagules and virtually fish larvae, respectively. The concept of "Cells of Ecosystem Functioning" (…" a holistic approach to environmental management, integrating the sea bottom with the water column") is fundamental for understanding the array of determinants underlying the pelagic habitats' connectivity and delimitation.

The zooplankton population values in the three pelagic habitats found in the current study were comparable with those recorded in the last years in the Romanian waters during the summer seasons (Muresan *et al.*, 2020; Boicenco *et al.*, 2018), envisaging a positive but fluctuating tendency of trophic zooplankton related to the overwhelming proportion of gelatinous species recorded in the previous decades (Shiganova *et al.*, 2014). The thermophilic filter-feeding species of cladocerans (*P. avirostris, P. tergestina*), copepods (*Acartia spp., C. ponticus*), *O. dioica,* and predator *P. setosa* made up the greatest bulk of the secondary pelagic production, which is in accordance with most studies performed in the Black Sea evincing the strong seasonal pattern of the zooplankton (BSC, 2019). Some researchers (Shiganova and Öztürk, 2010) highlighted a shift toward dominance of thermophilic species starting at an earlier phase due to sudden warming at the very beginning of the summer. Nevertheless, the driven mechanisms for ecological changes at the individual taxa level proved to be harder to predict than at the community level. According to Vereshchaka *et al.,* 2019, natural twofold-year periodicities characterize most holoplankton taxa and primary production of the Black Sea in long term and the response to environmental factors may be expressed in different ways. In turn, the *total mesozooplankton biomass* could be robustly and predictably linked to temperature and productivity, constituting, therefore, a more reliable trait to assess the changes in pelagic ecosystem quality status as well as their tendency, and temporal and spatial magnitude.

We used the zooplankton indicators proposed at the Black Sea level in the framework of MSFD (Magliozzi *et al.,* 2021a), to assess the quality status of the pelagic habitats in August 2021. In addition, an integrative index (the Integrative Mesozooplankton Index) has been derived aiming to facilitate the decision-making process of authorities concerning spatial planning and ecosystem services valuation. While each index may give particular insights into different structural and functional aspects of the pelagic ecosystem, such as eutrophication (*The biomass of N. scintillans*), productivity, food web balance (*The Mesozooplankton biomass*), or climate change and pollution (*The biomass of copepods*), the Integrative Mesozooplankton Index (IMI) encompasses the information of all three above. Based on the expert judgment method, three classes and GES threshold have been proposed to assess the pelagic habitats (the integration method used: percentage of indicators within limits (ICES, 2018) that considered also the criteria that stood at the basis of establishing the GES threshold according to the reference period).

One of the policy recommendations for the Black Sea pelagic habitats (Magliozzi *et al*., 2021) suggests that the combination of three main indicators such as phytoplankton biomass, total mesozooplankton biomass and Copepoda biomass, could be integrated using an averaging method and the overall D1C6 criterion (The condition of the habitat type, including its biotic and abiotic structure and its functions, is not adversely affected due to anthropogenic pressures) GES (of all the pelagic components) could then be defined by the one-out all-out integration rule. While we mostly agree with this approach, we deem that integration also of *Noctiluca* biomass will much add valuable contribution to GES assessment at regional level. Our study represents a first attempts to integrate the mesozooplankton indices proposed under the framework of MSFD (Moncheva and Boicenco, 2011). Further considerations should be given to establishing thresholds taking into consideration characteristic and variability of different pelagic habitats, by taking advantage of the new capabilities of remote sensing technologies and *in situ* continuous monitoring tools to provide reliable spatial and time coherent data on pelagic habitats.

The results of the GES assessment in August 2021 based on the three indices showed that in only **50%** of the transitional stations, **83%** of the coastal waters, and **70 %** of the marine water, the GES has been achieved. Among these, *The Biomass of copepods* revealed the worst ecological status, with only **43 %** of the stations that reached GES, followed by *The Mesozooplankton biomass* and *The biomass of N. scintillans*, with 78% and 87% of stations. Hence, although the use of the three indices to calculate IMI could satisfy the needs for integration according to the requirements of the MSFD, a higher number of indices could be also used in the assessment. To increase the IMI's accuracy and functionality, all five zooplankton indices (the Shannon diversity (H') index, and *Mnemiopsis biomass*, in addition to already considered indices) along with the phytoplankton and relevant abiotic variables (hydrological and physical-chemical parameters) could be integrated. It is worth noting that the Black Sea mesozooplankton diversity is quite poor (around 15-20 species) and may vary depending on season, sampling strategy and taxonomic resolution, therefore the Shannon index might not be always reliable. Moreover, according to the Commission Decision (EU) 2017/848, the MSFD indicators need to reflect clear pressure-response relationships (Magliozzi *et al.*, 2021b). At the European level, there is currently a lack of consistent assessment of D1C6 GES of pelagic habitats since fourteen out of sixteen indicators have an EU-wide scale of applicability but regional thresholds (Magliozzi *et al.*, 2021a). Still, there is a lot of debate on the most appropriate indices to be used to assess the quality of pelagic habitats at the European level (Ndah *et al.*, 2022). Most countries agreed that the assessment of plankton should include both vigor and organization of its environment, meaning the main lifeforms of plankton, and the primary production and its coupling to higher trophic levels (Scherer *et al.,* 2016). To this end, the functional traits would better reflect the status of pelagic habitats.

In the case of zooplankton indicators, these bottlenecks could be overcome, due to the increasing interest of scientists and the late requirements of the MSFD to include plankton among the descriptors of GES especially those related to biodiversity, food webs, and eutrophication (Gorokhova *et al.,* 2013; Gorokhova *et al.*, 2016; McQuatters-Gollop *et al.*, 2019). The most recent review by Ndah *et al.*, 2022 addressed critically the major challenges of developing new zooplankton indices and applying existing ones in the context of the MSFD. In the Black Sea, this work should continue with a more integrative approach and test of spatial and temporal reliability of existing indices.

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ANNEX

Table 1. Synopsis of zooplankton samples collected in August 2021

No. crt.	Station	Water body (Pelagic hab.)	Data	Latitude [degrees N]	Longitude [degrees E]	Depth (m)
1	P001	T	19-08-21	44.654	29.0418	13
2	SU01	T	17-08-21	45.068	29.74193	15
3	TZ18	C	13-08-21	43.990	28.72198	33
4	MA07	C	14-08-21	43.767	28.65637	35
5	EF02	C	13-08-21	44.070	28.66455	16
6	P005	C	18-08-21	44.577	29.22485	29
7	SG03	M	17-08-21	44.813	29.67063	37.3
8	CT05	M	14-08-21	43.974	29.5115	65
9	MA03	M	15-08-21	43.741	29.7378	70
10	SG05	M	18-08-21	44.590	30.10045	64
11	MID02	M	19-08-21	44.299	29.2292	43
12	CT04	M	19-08-21	44.082	29.03457	47
13	SG14	M	16-08-21	44.463	30.31143	79
14	SU04	M	17-08-21	44.901	30.45035	52
15	MA02	M	15-08-21	43.732	30.03447	118
16	MA11	M	14-08-21	43.758	29.06727	57.7
17	MA08	M	14-08-21	43.772	28.73513	45
18	P004	M	18-08-21	44.525	29.3421	41
19	P006	M	18-08-21	44.425	29.58408	55
20	SU03	M	17-08-21	45.044	30.05518	35
21	SG04	M	18-08-21	44.671	29.8137	53
22	SG06	M	16-08-21	44.331	30.51652	93
23	SG09	M	16-08-21	44.157	30.78477	149

Table 2. The composition and univariate indices of abundance of zooplankton populations (Davg, Bavg: average density and biomass; F%: frequency, DD%, DB%: dominance; WD, WB: significance ecological indices after density and biomass)

Taxa	Davg (ind.m-3)	Bavg (mg.m-3)	F%	DD%	DB%	WD	WB
Noctiluca scintillans (Macartney) Kofoid & Swezy, 1921	543.93	47.87	73.91	7.62	11.10	**23.73**	**28.65**
Penilia avirostris Dana, 1849	1439.81	50.39	95.65	**20.17**	11.69	**43.92**	**33.44**
Pseudevadne tergestina Claus, 1877	996.24	3.98	100.00	**13.95**	0.92	**37.36**	9.61
Evadne spinifera P.E. Müller, 1867	1009.47	4.04	100.00	**14.14**	0.94	**37.60**	9.68
Pleopis polyphemoides (Leuckart, 1859)	138.72	1.25	43.48	1.94	0.29	9.19	3.55
Acartia (Acartiura) clausi Giesbrecht, 1889 & *Acartia (Acanthacartia) tonsa* Dana, 1849	690.49	13.64	100.00	9.67	3.16	**31.10**	17.78
Pseudocalanus elongatus (Brady, 1865)	208.64	9.01	78.26	2.92	2.09	15.12	12.79

ANNEX

Tabel 2 (continued)

Taxa	Davg (ind.m-3)	Bavg (mg.m-3)	F%	DD%	DB%	WD	WB
Oithona davisae Ferrari F.D. & Orsi, 1984 & *Oithona similis* Claus, 1866	47.28	0.25	95.65	0.66	0.06	7.96	2.34
Centropages ponticus Karavaev, 1895	540.20	20.37	100.00	7.57	4.73	**27.51**	21.74
Calanus euxinus Hulsemann, 1991	138.10	97.60	73.91	1.93	**22.64**	11.96	**42.09**
Oikopleura (Vexillaria) dioica Fol, 1872	258.40	13.23	100.00	3.62	3.07	19.02	17.13
Parasagitta setosa (J. Müller, 1847)	319.23	146.73	95.65	4.47	**34.03**	**20.68**	**57.06**
Amphibalanus improvisus (Darwin, 1854) larvae	117.22	2.66	65.22	1.64	0.62	10.35	6.34
Bivalvia larvae	147.65	0.27	82.61	2.07	0.06	13.07	2.28
Gastropoda larvae	363.39	3.36	91.30	5.09	0.78	**21.56**	8.43
Polychaeta larvae	160.30	7.90	73.91	2.25	1.83	12.88	11.64
Ihtyoplankton	20.32	8.58	100.00	0.28	1.99	5.34	14.11
Average total trophic zooplankton (ind.m-3/ mg.m-3)	**7139.38**	**431.13**					

Table 3. Analysis of Similarities (ANOSIM) test of differences between unordered Pelagic habitats groups based on mesozooplankton average densities.

Tests for differences between unordered Pelagic hab. groups
Global Test
Sample statistic (R): 0.588
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from 1119195)
Number of permuted statistics greater than or equal to R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
1, 2	0.036	46.7	15	15	7
1, 3	0.637	1.3	153	153	2
2, 3	0.618	0.2	4845	999	1

Table 4. Analysis of Similarities (ANOSIM) test of differences between unordered Pelagic habitats groups based on mesozooplankton average biomass.

Tests for differences between unordered Pelagic hab. groups
Global Test
Sample statistic (R): 0.369
Significance level of sample statistic: 0.4%
Number of permutations: 999 (Random sample from 1119195)
Number of permuted statistics greater than or equal to R: 3

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
1, 2	0.393	13.3	15	15	2
1, 3	0.396	3.3	153	153	5
2, 3	0.357	1.9	4845	999	18