Present Ecological Status of the Main Benthic Habitats from the Romanian Black Sea Shelf (*Valeria Abaza, Camelia Dumitrache, Adrian Filimon, Oana Marin*)	"Cercetări Marine" Issue no. 49 Pages 74 - 91	2019

PRESENT ECOLOGICAL STATUS OF THE MAIN BENTHIC HABITATS FROM THE ROMANIAN BLACK SEA SHELF

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ABSTRACT

Benthic fauna on sedimentary habitats has been analysed on 133 samples collected in 43 stations between 2015 and 2018 from the national monitoring network at depths ranging from 5 to 100m. The aim of the analysis was the identification of the main communities inhabiting sandy, muddy and mixed habitats and the assessment of their ecological status. In the above-mentioned period, 113 zoobenthic species have been identified; also, distinct communities have been evinced at 5-10, 20-30, 35-57 and 70-100m, dominated by *Ampelisca sarsi* (Chevreux, 1888) (at 5-10m), *Melinna palmata* (Grube, 1870) and various bivalves such as *Chamelea gallina* (Linnaeus, 1758), *Abra prismatica* (Montagu, 1808), *Spisula subtruncata* (da Costa, 1778), *Anadara kagoshimensis* (Tokunaga, 1906) (at 20-30m), *Mytilus galloprovincialis* (Lamarck, 1819) and its associated fauna (at 35-57m) and *Modiolula phaseolina* (Philippi, 1844) (at 70-100m). For the environmental status assessment of sedimentary habitats based on zoobenthic communities, abundance values were used to calculate the species' richness, Shannon diversity index, AMBI index, and its five ecological groups, and multimetric index M-AMBI*(n). By applying the principle "One Out All Out" set out by the WFD, the sedimentary habitats on the Romanian shelf were assessed as non-GES.

In shallow waters (0-3m), in the Romanian southern area, from Navodari to Vama Veche (eleven sampling stations), benthic habitats dominated by key species (*Zostera noltei* Hornemann, 1832, *Cystoseira barbata* (Stackhouse) C.Agardh, 1820) and various macrophytes have been analysed in the same period (2015-2018) based on the Ecological Index (EI) while the ecological status of benthic habitats dominated by macrophytes and marine phanerogams were assessed.

Key words: benthos, macrophytes, habitats, MSFD, ecological status

AIMS AND BACKGROUND

Considering the unique peculiarities of the Black Sea, studies carried out in 1950-1960 on the Romanian shelf evinced a rich marine biota, continuously expanding as new species were discovered and described. As a result of the economic development of the coastal zone, marine ecosystems started to undergo structural and functional changes, reflected in an increasingly advanced state of deterioration manifested by reduction of specific diversity, simplification of trophic network, degradation and even loss of benthic habitats. (Petran, 1997; BSC, 2007, 2008). Currently, although physical and biological parameters of marine environment show signs of improvement, the overall status is still far from the reference conditions (similarly to the 1960s), recent studies showing that the ecosystem is unlikely to return to what it was, considering actual climate changes and anthropogenic interventions in the marine and coastal environments (Nicolaev, Zaharia, 2016, Lazar *et al.*, 2018, Teaca *et al.*, 2019). In the same time, the ecological assessment of two designated marine protected areas in the Romanian Black Sea showed that despite that benthic habitats were in GES, according to AMBI index, the macrozoobenthic communities were dominated mainly by disturbance-tolerant species and second-order opportunistic species, (Begun *et al.*, 2018).

Both phytobenthic and zoobenthic communities represent important components of benthic habitats, whose degradation influence other biotic components, so continuous annual monitoring programme is necessary in order to capture any changes related to their composition and quantitative structure. The main role of macrophytes is to provide areas favourable for the development of life in shallow waters (feeding, reproduction and shelter for numerous marine organisms, either invertebrates or vertebrates).

Presently, both MSFD and Commission Decision 848/2017 require Member States to define good environmental status of marine waters under their jurisdiction based on specific criteria and methodological standards. For benthic macroinvertebrates within the geographic intercalibration process between Bulgaria and Romania, the M-AMBI*(n) index (Sigovini *et al.*, 2013) has been tested and used in order to assess the ecological status of the coastal waterbody (Todorova *et al.*, 2015) and transitional waters (Abaza *et al.*, 2016) for the purpose of WFD and for marine broad habitat types in the MSFD scope (Abaza *et al.*, 2018). The assessment concept is based on the principles that disturbance –sensitive taxa decrease, while tolerant and opportunistic species increase along the increasing pressure gradient, coupled with decrease in species richness and evenness of distribution. These two aspects of the invertebrate community change are reflected by AMBI and S/H’ respectively, combined in the composite index M-AMBI*(n) (Todorova *et al.*, 2015).

In order to define good environmental status of marine coastal waters based on the macrophytes communities, the Ecological Index (EI) was applied (Marin O. *et al.*, 2015; Berov D. *et al.*, 2018). The general idea is that in high eutrophication conditions, macrophytes communities present a very simplified and patchy structure, with monospecific character, and a dominance of tolerant and opportunistic species.

The aim of the present paper represents the identification of the main communities inhabiting sedimentary habitats for macroinvertebrates and marine phanerogams, main flora associations on hardbottom habitats, and the assessment of their ecological status.

EXPERIMENTAL

The phytocoenosis have been monitored annually, during warm season, between 2015 – 2018, along eleven sampling stations from Navodari to Vama Veche (Navodari, Pescarie, Constanța North, Cazino Constanța, Agigea, Eforie Sud, Tuzla, Costinești, Mangalia, 2 Mai, Vama Veche - Fig.1.), in coastal waters, at depths ranging from 0 to 3m, the maximum developmental area for macroalgae belts. A total number of 322 samples was collected.

After 5 m, the macrophytes have a patchy distribution, not relevant for a real assessment of the ecological status of the benthic habitats based on this biological element. The methodology involves collecting samples from the area with maximum specific diversity. A set of three samples were collected (using the square method with a 20/20 cm side frame) in each depth range (0-1m, 1-2m, 2-3m). One special situation is represented by key species - *Cystoseira barbata* (Stackhouse) C.Agardh, 1820, *Coccotylus truncatus (Pallas)* M.J.Wynne & J.N.Heine, 1992, *Zostera noltei* Hornemann, 1832 - where only one sample was collected at each depth range, in order to minimize the impact on them. *C. barbata* (Stackhouse) C.Agardh, 1820 and *Z. noltei* Hornemann, 1832 were sampled between 0 - 3m, while *C. truncatus (Pallas)* M.J.Wynne & J.N.Heine, 1992 was sampled between 6 – 8 m.

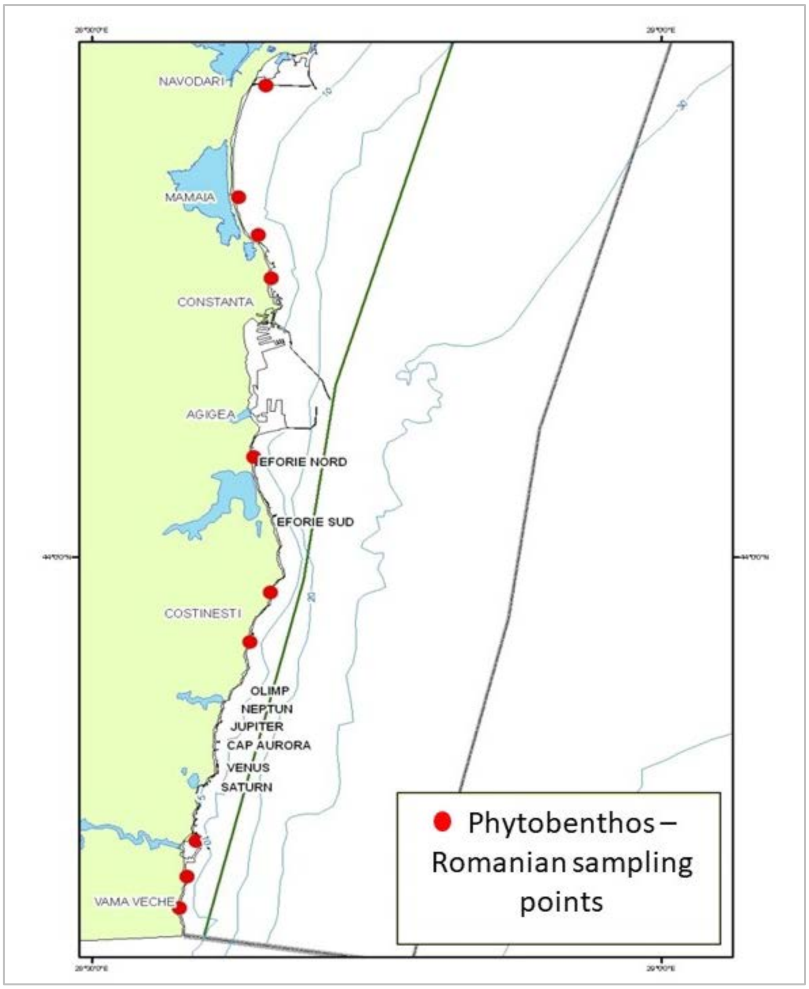


Fig.1. Sampling stations for macrophytobenthos along the Romanian coast.

After determining the wet biomass of each species per square meter (by multiplying the obtained value by the 25 coefficient), the ecological index (EI) is applied. The ecological status of benthic habitats dominated by macroalgae along the Romanian Black Sea coast was assessed by referring to the broad habitat types and their sub-types. The condition of each broad habitat is established by referring to each sampling station included in that habitat, ecologically assessed using EI. The resulted values for each station were averaged, the final value reported to the threshold presented above (EI must be higher than 6), resulting in the ecological status of each habitat.

Each identified species was included in ecological groups according to its tolerance to environmental conditions, namely ESG IA, ESG IB, ESG IC – perennial species indicator of less eutrophic areas, and ESG IIA, ESG

IIB, ESG IICa, ESG IICb - opportunistic species able to thrive in eutrophic areas with a high reproductive capacity (eg. *Ceramium* Roth, 1797, *Cladophora* Kützing, 1843, *Ulva* Linnaeus, 1753). Main criteria in differentiating the species into sensitivity groups was species morphology, biology and growth rates, as well as observational and experimental evidence of their sensitivity to eutrophication in the specific conditions of the Black Sea.

The average biomass of sensitive (ESGI) and tolerant (ESGII) species from all samples collected from replicate transects is calculated. The index is expressed as the proportion of sensitive and tolerant species average biomasses at each transect. As a value of EI, the biomass proportion of the most sensitive group is taken into consideration. EI takes values in the range of 0-10, divided in five classes (Table 1). The proportion of each ESG group within the two main groups ESG I and ESG II was corrected with a coefficient. The criteria for this correction were distribution along the eutrophication gradient, phenotypic plasticity and growth rate. Weight coefficients were defined for different subgroups as follows:

ESG IA–coef=1
ESGIB-coef=0.8
ESGIC-coef=0.6
ESGIIA-coef=0.6
ESGIIB-coef=0.8
ESGIIC-coef=1

To calculate the value of EI the following rules and formulas are applied:
EI bad (0-1)=[ESGIICa/ESGII], ESGII=ESGIICa+ESGIICb (non-GES)
EI bad(1-2)=[(ESGIIA/ESGII)*0.6+(ESGIIB/ESGII)*0.8]+1 (non-GES)
EI poor (2-4)=
5*[(ESGIA/ESG)*1+(ESGIB/ESG)*0.8+(ESGIC/ESG)*0.6]+2 (non-GES)
EI high, good, moderate=
(4-10)=10*[(ESGIA/ESG)*1+(ESGIB/ESG)*0.8+(ESGIC/ESG)*0.6] (GES)

The EI obtained value is related to the Table 1 limits and the ecological status for the respective habitat is defined (Good or Bad ecological status).

Table 1. EI target values for defining the ecological status

Biomass proportions of sensitive and tolerant species	EI	Ecological status
80-100 % ESGI	7.8 - 10	Good (GES)
60-80 % ESGI	6 - 7.8	
40-60 % ESGI	4 - 6	Bad (non-GES)
0-40 % ESGI	2 - 4	
0 % ESGI	< 2	

Zoobenthic communities have been studied between 2015 and 2018 on 133 samples collected from 43 stations of the national monitoring network from Sulina to Vama Veche at 5-100m depth (Fig.2). Sampling was undertaken on sedimentary bottoms according to the common methodology for the Black Sea region (Todorova, Konsulova, 2005), using a Van Veen grab on a surface

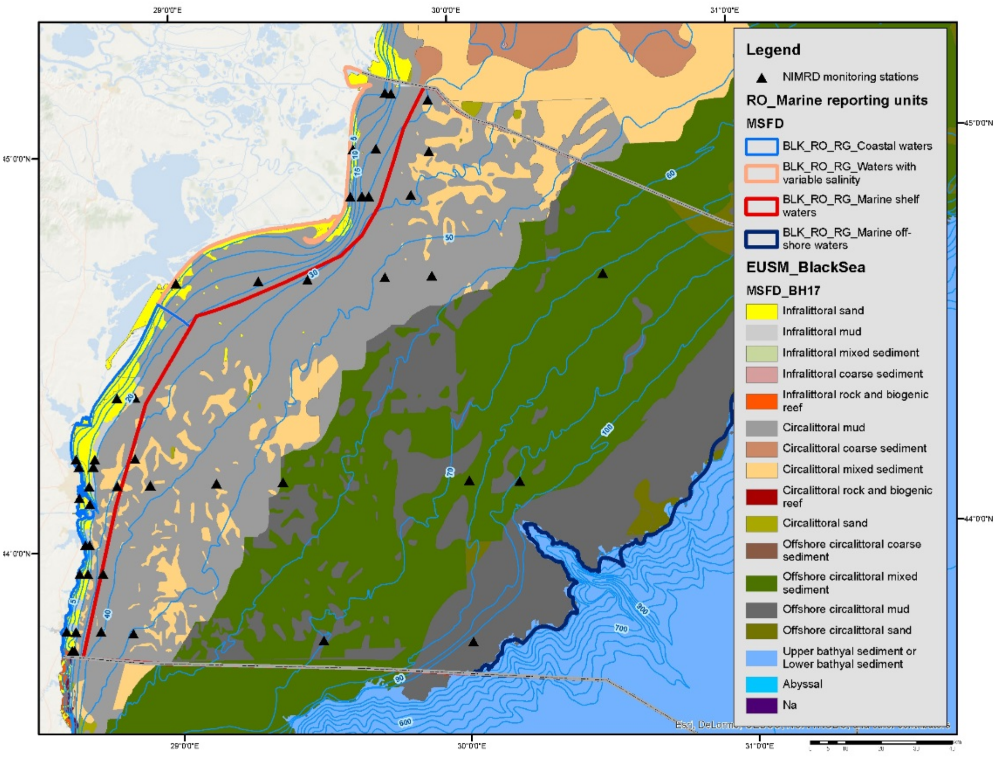


Fig. 2. Zoobenthos sampling network in the Romanian shelf area.

of 0.1m^2 with a frequency of once per year. Collected samples were stored in plastic bags aboard the sampling vessel and buffered with 40% formaldehyde. In the laboratory, they were washed using a set of stainless-steel gauze sieves with a mesh size of 1.0 × 1.0 mm and 0.5 × 0.5 mm. After the organisms were determined under stereomicroscope, the abundance (ind./m^2) and biomass (g/m^2) were calculated. Based on abundance data, species richness (S), Shannon diversity index (H'), and AMBI index were calculated, using the AMBI software (http://ambi.azti.es). Averaging the normalized value of S, H' and AMBI, the multivariate index M-AMBI*(n)(Sigovini *et al.*, 2013) has been calculated in order to assess the ecological status of the broad habitat types (physical habitats) identified in the Romanian marine waters. Table 2 presents the boundaries between GES and non-GES for the main sedimentary broad habitat types identified on the Romanian Black Sea shelf.

Table 2. EQR and M-AMBI*(n) values defined for the main sedimentary broad habitat types on the Romanian Black Sea shelf

Class boundaries	**Variable salinity and *Modiolula* benthic habitats**		**Coastal and *Mytilus* biogenic reefs benthic habitats**		**Ecological status**
	EQR	**M-AMBI*(n)**	**EQR**	**M-AMBI*(n)**	
High/Good	0.90	0.81	0.90	0.90	Good (GES)
Good/Moderate	0.68	0.61	0.68	0.68	
Moderate/Poor	0.45	0.41	0.45	0.45	Bad (Non-GES)
Poor/Bad	0.23	0.21	0.23	0.23	

The identification of broad habitat types was based on statistical analysis of both macrophytobenthos biomass and zoobenthos abundance data using PRIMER v.7 software.

RESULTS AND DISCUSSION

The infralittoral phytobenthic associations have been analysed on 322 samples collected from the national monitoring network between 2015-2018. Two benthic broad habitat types and four sub-types have been delimiting into the infralittoral community (Commission Decision (EU) 2017/848 of 17 May 2017) (Fig.3):

Benthic broad habitat types:

- ✓ Infralittoral rock and biogenic reef
- ✓ Infralittoral mud

Sub-types of broad habitat types:

- ✓ Infralittoral rock with photophilic algae: as a sub-type of the Infralittoral rock and biogenic reef habitat
- ✓ *Cystoseira* Habitat: as a sub-type of the Infralittoral rock and biogenic reef habitat
- ✓ *Phyllophora* Habitat: as a sub-type of the Infralittoral rock and biogenic reef habitat
- ✓ *Zostera* Habitat: as a sub-type of the Infralittoral mud habitat.

The sub-type *Cystoseira* habitat was found towards the southern coast, along Mangalia - Saturn - 2 Mai - Vama Veche, whilst the one formed by the marine phanerogam *Zostera noltei* Hornemann, 1832 has a more fragmented distribution, in the northern part (Navodari) and in the southern part (Mangalia). Among *Phyllophora* species, only two species can be found along the Romanian coast nowadays: *Coccotylus truncatus* (Pallas) M.J.Wynne & J.N.Heine, 1992 and *Phyllophora crispa* (Hudson) P.S.Dixon, 1964. *C. truncatus* (Pallas) M.J.Wynne & J.N.Heine, 1992 forms a special habitat in an extremely small area, with a punctiform distribution, at Constanta North, at depths between 6 to 7m (Fig.1). *P. crispa* (Hudson) P.S.Dixon, 1964 has an even smaller distribution, only in the northern part of the Romanian Black Sea coast, at Sf. Gheorghe.

Regarding the ecological status of these sub-types of particular ecological importance for marine environment, it can be said that they have reached the good ecological status between 2015 - 2018, with EI values higher than the threshold value (meaning that EI was higher than 6), as follows:

- ✓ for *Cystoseira* Habitat, the EI value was 7.73,
- ✓ for *Phyllophora* Habitat, the EI value was 7.25,
- ✓ for *Zostera* Habitat, the EI value was 7.62.

The exception was the ecological status of *Phyllohora* habitat, but only for 2018, when the good ecological status was not reached (Fig.4c). This is only a particular situation that requires continuous monitoring in order to confirm or deny this aspect. *Phyllophora* Greville, 1830 species are very sensitive, being considered Critically Endangered (CR) according to the IUCN criteria along the Romanian Black Sea coast.

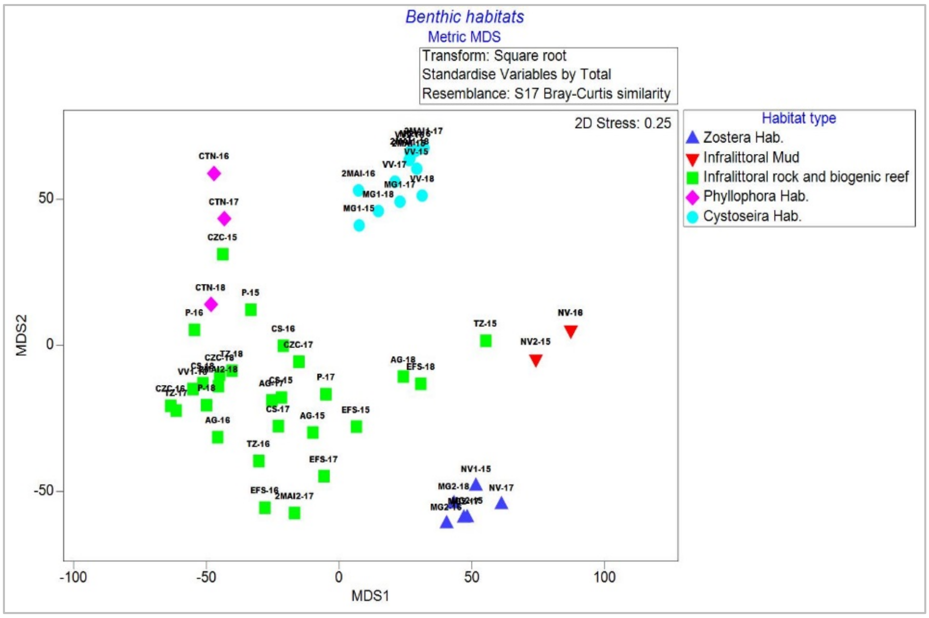
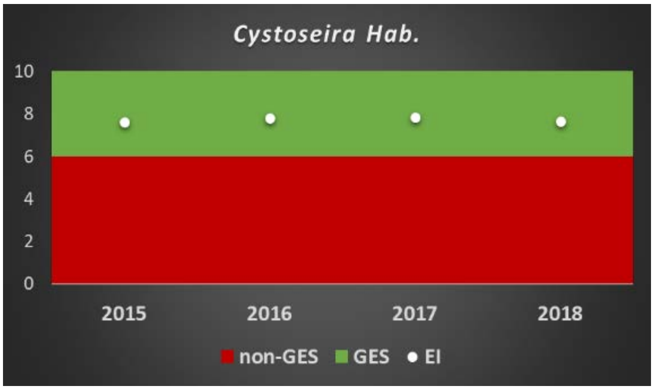
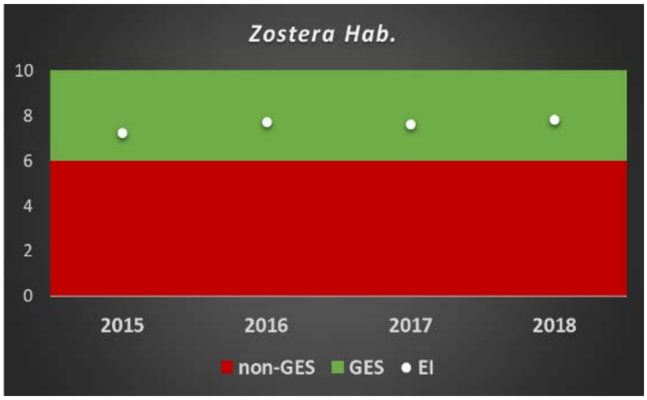


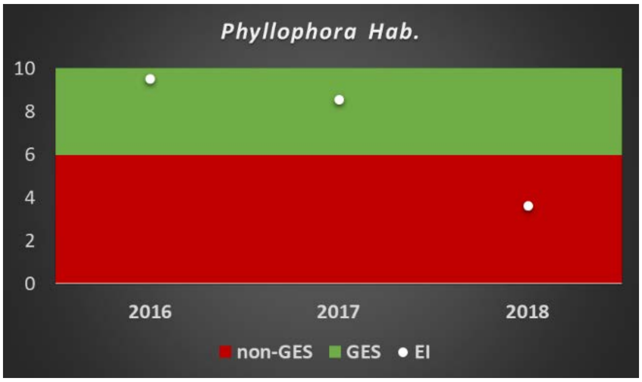
Fig. 3. Distribution of macrophyte habitat types along the Romanian Black Sea coast.



a) *Cystoseira barbata* (Stackhouse) C.Agardh, 1820



b) *Zostera noltei* Hornemann, 1832

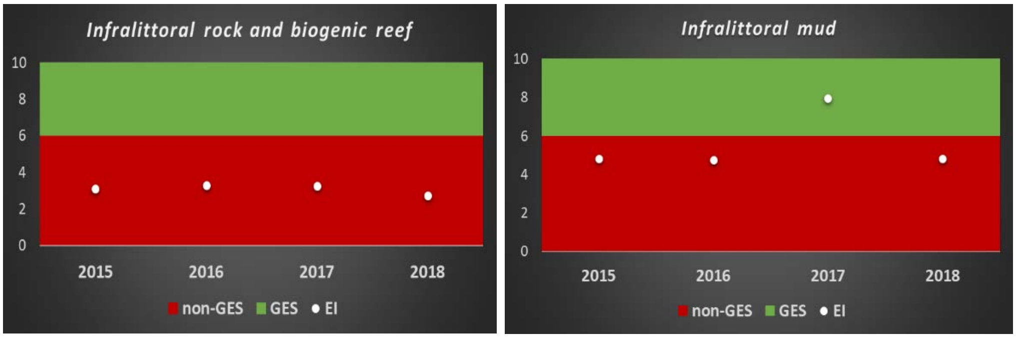


c) *Phyllophora brodiaei* (Pallas) M.J.Wynne & J.N.Heine, 1992

Fig. 4. Ecological assessment of the subtypes *Cystoseira* Habitat (a), *Zostera* Habitat (b) and *Phyllophora* Habitat (c) between 2015 – 2018 for *Cystoseira* and *Zostera* and between 2016 – 2018 for *Phyllophora*.

The broad habitat Infralittoral rock and biogenic reef was identified in ten sampling stations, of which only three have been assessed as GES, so only 30% of the stations from this habitat are considered to be in a good ecological condition, with EI values higher than the threshold value 6 (Fig. 5a). The final evaluation for the period 2015 - 2018 showed that the broad habitat Infralittoral rock and biogenic reef did not reach the good ecological status (the EI value was 3.10).

The other broad habitat, Infralittoral mud, was identified at only two sampling stations, namely Năvodari and Mangalia. Only at Mangalia is this habitat in good ecological status, with EI values higher than 6. The global assessment for the considered period showed that this habitat did not reach the good ecological status (the EI value was 5.60) (Fig. 5b).



a) Infralittoral rock and biogenic reef **b) Infralittoral mud**

Fig. 5. Ecological assessment of the Infralittoral rock and biogenic reef broad habitat types (a) and Infralittoral mud (b) between 2015 – 2018.

The phytobenthic associations' qualitative analysis showed a pronounced uniformity regarding the specific diversity on rocky and muddy habitats, manifested by the presence of a small number of algal species. The opportunistic species (*Ulva* Linnaeus, 1753, *Cladophora* Kützing, 1843, *Ceramium* Roth, 1797) dominate all habitats, both as associated species and as epiphytes within the habitat sub-types presented above (*Cystoseira*, *Phyllophora* and *Zostera*) (Fig.6).

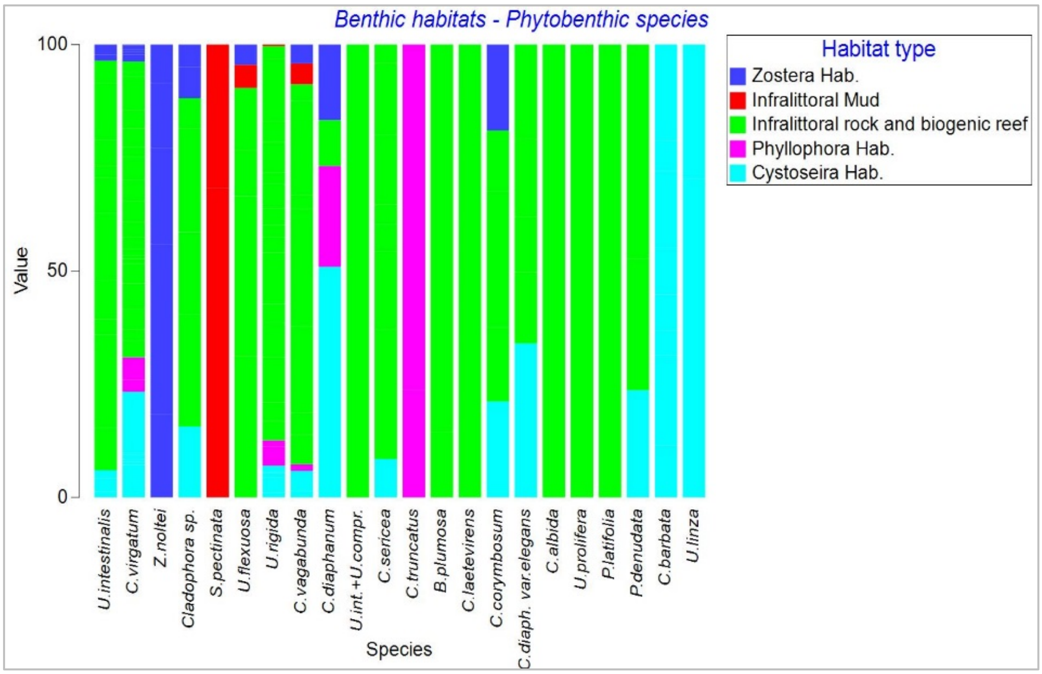


Fig. 6. List of phytobenthic species identified between 2015 and 2018 per habitat.

On Romanian continental shelf, 113 macrozoobenthic species belonging to numerous taxonomic groups: Polychaeta, Mollusca, Crustacea, Coelenterate, Turbellaria, Nemertina, Halacarida, Phoronida, Echinodermata, Tunicate have been identified at depths of 5-100m. According to Bacescu *et al.* (1971), some species may occur at various depths between 0 and 120m, (e.g. the *Ampelisca sp.* amphipods) whilst others occur at specific depths and sediment types (e.g. bivalves *Lentidium mediterraneum* (O.G. Costa, 1830) at 3-15m depth on fine sands, *Modiolula phaseolina* (Philippi, 1844) at 50-120m depth on grey muds mixed with various dead shells). According to Commission Decision (EU) 2017/848, the following broad habitat types (EUNIS level 2) have been identified in the Romanian Black Sea waters on sedimentary bottoms:

- ✓ Infralittoral sand (MB5)
- ✓ Infralittoral mud (MB6)
- ✓ Circalittoral sand (MC5)
- ✓ Circalittoral mud (MC6)
- ✓ Offshore circalittoral mixed sediments (MD4)
- ✓ Offshore circalittoral mud (MD6)

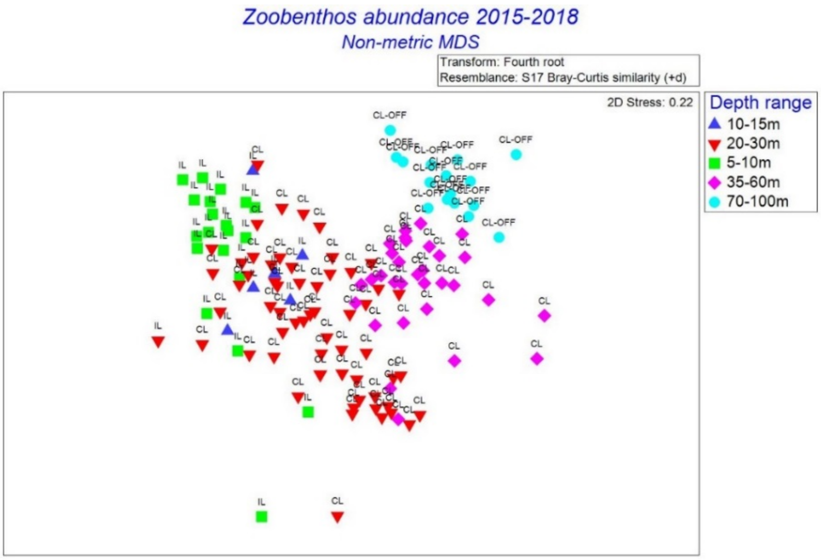


Fig. 7. Distribution of sedimentary broad habitat types on depth range.

Macrozoobenthic communities were distributed differently at various depths according to dominant species both in abundance and biomass (Fig.7). Benthic communities on infralittoral sediments are quite similar regardless of the analysed waterbody: variable salinity or coastal. The difference between the two waterbodies consists in the abundance and occurrence of the *Lentidium mediterraneum* (O.G. Costa, 1830) bivalve, benthos distribution being influenced by sediment nature rather than salinity variation (Fig.8).

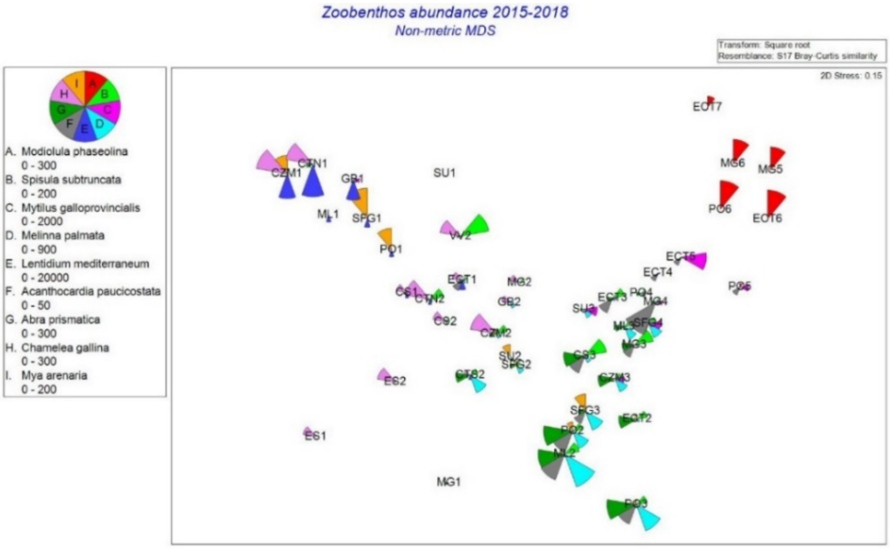


Fig. 8. Distribution of the main zoobenthic communities on the sedimentary broad habitat types on the Romanian Black Sea shelf.

Similarly, at a depth of 20m benthic communities dominated by polychaetes and bivalves such as *Chamelea gallina* (Linnaeus, 1758), *Spisula subtruncata* (da Costa, 1778) or *Anadara kagoshimensis* (Tokunaga, 1906) occur, although *Chamelea gallina* is well represented on infralittoral sands, especially in the southern area (south of Constanta) (Fig.6). On circalittoral sediments there are transitional communities to circalittoral mud with biogenic reefs of *Mytilus galloprovincialis* (Lamarck, 1819) or mixed with those at a depth of 20-30m; here, benthic communities are dominated by bivalves such as *Acanthocardia paucicostata* (G.B. Sowerby II, 1834), *Abra prismatica* (Montagu, 1808) (north of Constanta), *Spisula subtruncata* (south of Constanta), associated with a rich polychaete fauna dominated by *Melinna palmata* (Grube, 1870) and *Nephtys hombergii* (Savigny in Lamarck, 1818). Between 35 and 55-60m, a typical community of *Mytilus galloprovincialis*, forming biogenic reefs occurs, besides polychaetes such as *Polydora cornuta* (Bosc, 1802), *Prionospio cirrifera* (Wirén, 1883), *Heteromastus filiformis* (Claparède, 1864), *Nephtys hombergii* and *Terebellides stroemii* (Sars, 1835). Deeper than 55-60m, circalittoral grey muds, mixed with bivalve dead shells shelter the community dominated by the small *Modiolula phaseolina* (Philippi, 1844) bivalve, in association with fauna specific to deep sea sediments.

Based on the multimetric index M-AMBI*(n) values, ecological status of the broad habitat types identified in each water body was assessed for the period 2015-2018, establishing to the possible extent, its evolution trend.

In the waterbody with variable salinity situated in the northern part of the Romanian Black Sea, influenced by the riverine inflow of the Danube, both infralittoral sands and circalittoral mud were in GES, although no clear trend can be observed (Fig.9). Ecological status was obtained by averaging the values of M-AMBI*(n) index in all stations with the same habitat type.

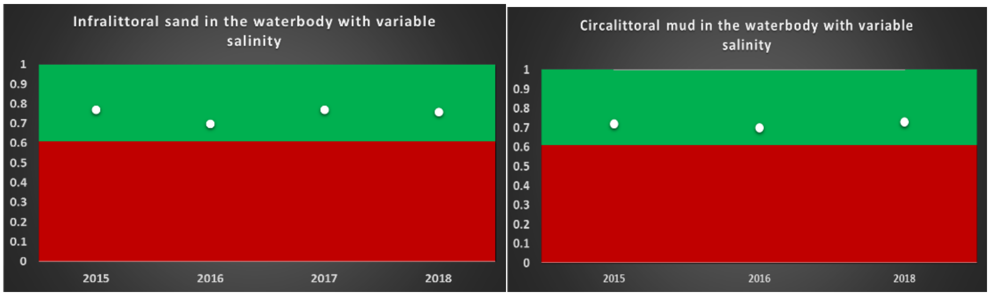
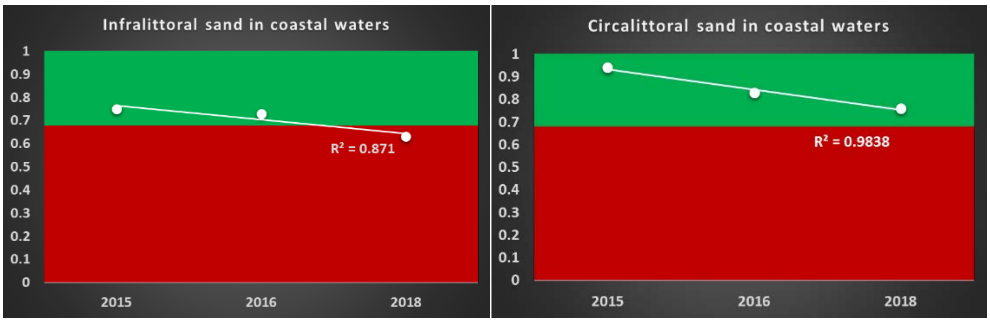


Fig. 9. Interannual changes of ecological status of broad habitat types in waterbody with variable salinity along the Romanian shelf of the Black Sea.

Following the same method, in the coastal waterbody, the three broad habitat types identified indicate different ecological status (Fig.10). Thus, infralittoral sands were in bad ecological status (non-GES), while circalittoral soft-bottom habitats (sand and mud) were in good ecological status (GES). For sandy bottoms, either infralittoral or circalittoral, a clear decreasing trend in ecological status can be observed, while the muddy habitats' status was stable (Fig.10).



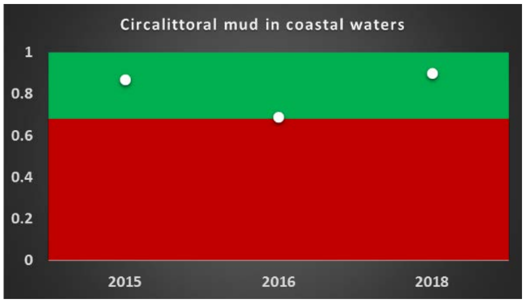


Fig. 10. Interannual changes of ecological status of broad habitat types in coastal waterbody along the Romanian shelf of the Black Sea.

In marine waters we identified circalittoral mud with *Mytilus galloprovincialis* biogenic reefs at a depth of 35-55m, and circalittoral mixed sediments with *Modiolula phaseolina* and its associated fauna at a depth of 55-100m. Both are in good ecological status according to averaged values of the M-AMBI*(n) index, although some of the stations of *Mytilus* biogenic reefs habitat were in bad ecological status. The evolution trend was found to be stable in the four-year analysis (Fig.11).

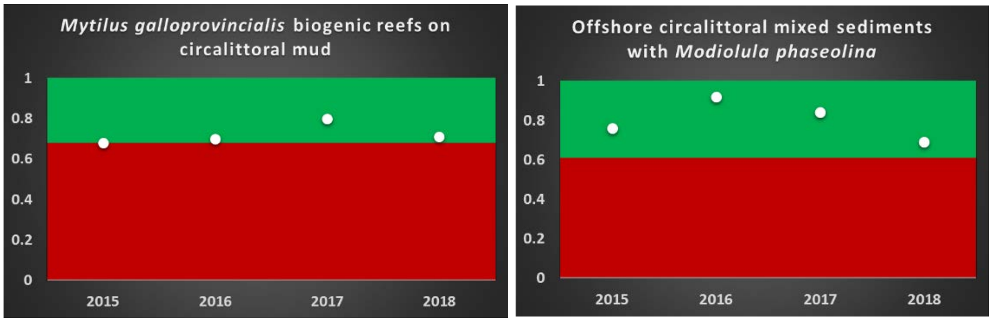


Fig. 11. Internannual changes of ecological status of broad habitat types in marine waters of the Romanian Black Sea shelf.

The hardbottom habitats were assessed only for the macrophytobenthic communities; for those inhabited by benthic invertebrates, the communities were not determined, and therefore no thresholds for GES were established. Our future efforts shall be focused on this very important issue.

CONCLUSIONS

For the Romanian Black Sea coast, within the infralittoral community in the coastal waterbody, two broad habitat types and four subtypes were identified and assessed for ecological status based on the macrophyte biological element. The Infralittoral rock and biogenic reef broad habitats and Infralittoral mud have been assessed as non-GES between 2015 and 2018. In contrast, the sub-types of the broad habitats formed by the key species *Cystoseira barbata* (Stackhouse) C.Agardh, 1820, *Coccotylus truncatus* (Pallas) M.J.Wynne & J.N.Heine, 1992 and *Zostera noltei* Hornemann, 1832 were assessed as GES during the same period.

Sedimentary broad habitat types in three waterbodies (variable salinity, coastal and marine) were identified by analysing their associated macroinvertebrate fauna. More data analysis is necessary in order to better establish the benthic habitat subtypes based on the biological communities and map them, accordingly.

Based on the averaged values of M-AMBI*(n) per habitat type, most of the sedimentary broad habitat types were assessed with a good ecological status, except for the infralittoral sand in coastal waters, which were in bad ecological status. The evolution trend in the four-year study were either decreasing (coastal sediments) or stable (variable salinity and marine waterbodies habitats). Considering the macrozoobenthic communities inhabiting the identified sedimentary broad habitats, and continuous data collection, further work is necessary to revise/renew the GES thresholds and identify other indicators to better characterise both their ecological status and future trends.

Hardbottom habitats' assessment was based on macrophytobenthic communities only; in those inhabited mostly by benthic invertebrates, the communities were not determined; therefore, no thresholds for GES were established. Our future efforts shall be focused on this very important issue, as well on identification of EUNIS level 3-4 habitats and GES thresholds.

Acknowledgement. This research has been carried out with financial support from the NUCLEU Programme (INTELMAR), funded by the Ministry of Education and Research, project no. PN 19260202.

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