

CURRENT ECOLOGICAL STUDY OF HARD SUBSTRATE HABITATS FROM COASTAL WATERS OF THE ROMANIAN BLACK SEA AREA

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Abstract

Benthic communities are considered excellent indicators of the ecological status of the marine environment. This study aims to present the results from the ecological analysis of benthic communities from infralittoral hard substrate habitats in Romanian coastal waters and provide updated information on the current structure and distribution of flora and fauna communities. During 2020-2021, macroalgae samples were collected from Pescărie - Vama Veche area, at depths between 0 and 8 m, while the zoobenthic samples were collected from Tuzla - Mangalia area in the bathymetric interval 2 and 10 m. The ecological evaluation of the phytobenthic communities was performed by applying the multiparametric Ecological Index, whilst the zoobenthic ecological study was accomplished based on the synecological analysis. Broad habitat Infralittoral rock and biogenic reef with its related sub-types: Upper-infralittoral rock dominated by *Cystoseira barbata*, Upper infralittoral rock with *Coccotylus brodiei*, Upper infralittoral rock with variable annual green and red macroalgae and Infralittoral rock dominated by Mytilids, were analysed. Upper infralittoral rock with variable annual green and red macroalgae did not achieve good ecological status in the last two years, while the habitats formed by the key species *C. barbata* and *C. brodiei* have been found in good ecological status. Zoobenthic communities' structure and distribution showed that *Mytilus galloprovincialis* community was dominant, constantly accompanied by *Mytilaster lineatus*, whose numerical dominance was highest between 2 and 3 m depth. Regarding the algae communities, quantitatively dominant were the green algae of the genera *Ulva* and *Cladophora* at most sampling stations.

Keywords: MSFD, macroalgae, zoobenthos, hard substrate habitats, good ecological status.

1 INTRODUCTION

Benthic marine habitats illustrate the natural diversity of the marine environment and are considered key elements in assessing the ecological status of the marine environment. The hard substrate from the southern part of the Romanian Black Sea coast occupies small areas, respectively 0.3% of the total area of the Romanian continental shelf, being represented by a discontinuous band of submerged rocky platforms, interrupted by sandy beaches. The width of these areas is variable, ranging from a few tens of meters to 4 km and a maximum depth of 28 m, up to the lower limit of the rocky platforms. Macroalgal communities are an important part of benthic hard substrate habitats, with a major role in transforming lifeless zones into suitable areas for the development of marine life. As well, the zoobenthic communities have an important ecological role, due to their diversity and structure. The most important ecological role of benthic fauna is of natural biofilter, since almost all sessile organisms and many of the moving species' feed by filtration, retaining organic and

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inorganic suspensions, thus ensuring water quality from the coastal area [1]. The current ecological study was focused on the broad habitat type Infralittoral rock and biogenic reef with its related subtypes: Upper infralittoral rock with variable annual green and red macroalgae (1-10 m), Upper infralittoral rock dominated by *Cystoseira barbata* (3-10 m), Upper infralittoral rock with *Coccotylus brodiei* (1-10 m) and Infralittoral rock dominated by Mytilids (*Mytilus galloprovincialis*, *Mytilaster lineatus*). Upper infralittoral rock with variable annual green and red macroalgae and Infralittoral rock dominated by Mytilids are considered common habitats, with a continuous distribution along the Romanian Black Sea coast. In contrast, Upper infralittoral rock dominated by *C. barbata* and Upper infralittoral rock with *C. brodiei* have a patchy distribution, in the southern part (from Jupiter to Vama Veche – *C. barbata* habitat) and near Constanta city (*C. brodiei* habitat), respectively. All these hard substrate habitats hold extremely diverse flora and fauna, including different endangered or protected species.

2 METHODOLOGY

Along the Romanian Black Sea coast, the monitoring of the phytobenthic communities was performed during warm seasons 2020 and 2021, based on samples collected from Pescărie to Vama Veche (Fig. 1). The sampling stations were selected due to their main peculiarities, a true mosaic of benthic habitats occurring in these areas (especially in the southern part of the Romanian Black Sea shore).

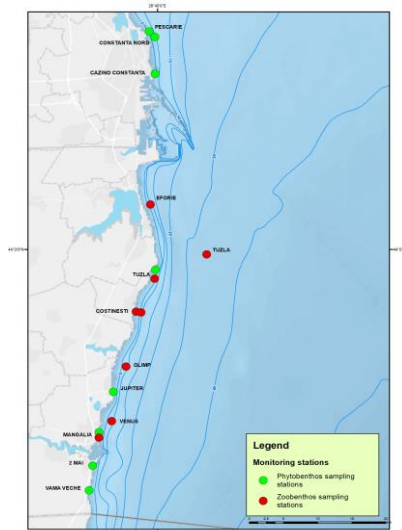


Figure 1. Sampling stations map

3 replicate samples were collected from the rocky infralittoral community, from each depth gradient (0 to 8 m), using a square frame of 20x20 cm. The fresh biological material was subsequently qualitatively (algal material observations) and quantitatively (calculation of fresh biomass) analysed [2], [3]. The ecological evaluation of hard substrate habitats (based exclusively on macroalgal communities) was performed by applying Ecological Index (EI). This is a multiparametric index based on the proportion of sensitive and eutrophication tolerant species and includes aspects of wet biomass and specific diversity. The basic principle consists in reporting

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the EI obtained values to a "threshold value" (considered to be 6), thus defining the ecological status as good (GES) or bad (non-GES) [4], [5]. Zoobenthos sampling stations were established in Tuzla, Eforie, Costinesti, Olimp, Venus, Mangalia, at 2m, 2.5 m, 3m, 6m, 7m, 8m and 10m depths (Fig.1). Three replicates from each station were collected using a square frame of 10x10 cm [6]. The scuba diver was able to observe the nature of the substrate and the degree of its coverage with flora and fauna (*visual census by scientific diving*). The collected material was placed in plastic containers, fixed with 4% formaldehyde, and labeled. In the laboratory, the samples were washed using 1 mm and 0.5 mm mesh size sieves. Each fraction of the sample was analyzed under a stereomicroscope, and the organisms identified to the lowest possible taxonomic level. The quantitative analysis of zoobenthos consisted in assessing the number of organisms as density (ind/m²) and biomass (g/m²). For ecological evaluation, a synecological analysis was performed. This analysis allows the identification of the species with the most significant contribution to the ecosystem in terms of function and energy exchanges with the environment. Also establishes the number of characteristic and accidental species. Dominance D% (relative abundance), constancy (C%) and the ecological significance index (W, Dzuba index, that reflects the relationship between structural (constancy, C) and productive (dominance, D) indices, showing the position of a species in a biocoenosis), were used as analytical ecological indices [7]. Statistical analysis was performed using PRIMER 7 (v.7.0.17) and XLSTAT 2021.3.1. [8].

3 RESULTS

3.1 Macroalgal Communities From Marine Habitats With Hard Substrate

In the Upper infralittoral rock with variable annual green and red macroalgae habitat, in both 2020 and 2021 (summer seasons), the dominance of the photophilic association *Ulva - Cladophora - Ceramium*, consisting exclusively of opportunistic species generating algal deposits, was noticed. During 2020-2021, green algae of the genera *Ulva* and *Cladophora*, compared to red algae, registered a more abundant development during summer season, with wet biomass values exceeding 2000 g/m². In comparison, in 2021, *Ulva* species showed slightly higher fresh biomass values compared to the same period of the previous year; in 2021, the maximum biomass value was 2,300 g/m², while in 2020, was 2,100 g/m². Regarding *Cladophora* species, the situation was the opposite, with lower biomass values in 2021 (2,500 g/m²) compared to 2020 (2,900 g/m²). These species, although present at most sampling stations, developed differently, in accordance with the environmental conditions (rocky substrate distribution, water transparency) and nutrients inputs (mainly nitrogen and phosphorus). PCA analysis showed that in both analysed years, *Ulva* species (especially *U. rigida* and *U. intestinalis*) developed more abundantly mainly in the southern part of the Romanian Black Sea coast, especially at 2 Mai – Vama Veche. *Cladophora* ssp. developed abundantly during summer 2020 in Pescărie area (a maximum recorded value of this period was 2,900 g/m²), Tuzla and 2 Mai, while in 2021, high biomasses were observed only in Mangalia (maximum recorded value 2,500 g/m²). Among the red algae, *Ceramium* ssp. (especially *C. virgatum*), although with no considerable quantities in the last years, showed a more pronounced

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development at Mangalia, in 2020 (100 g/m²) and Casino Constanța, in 2021 (250 g/m²) (Fig. 2).

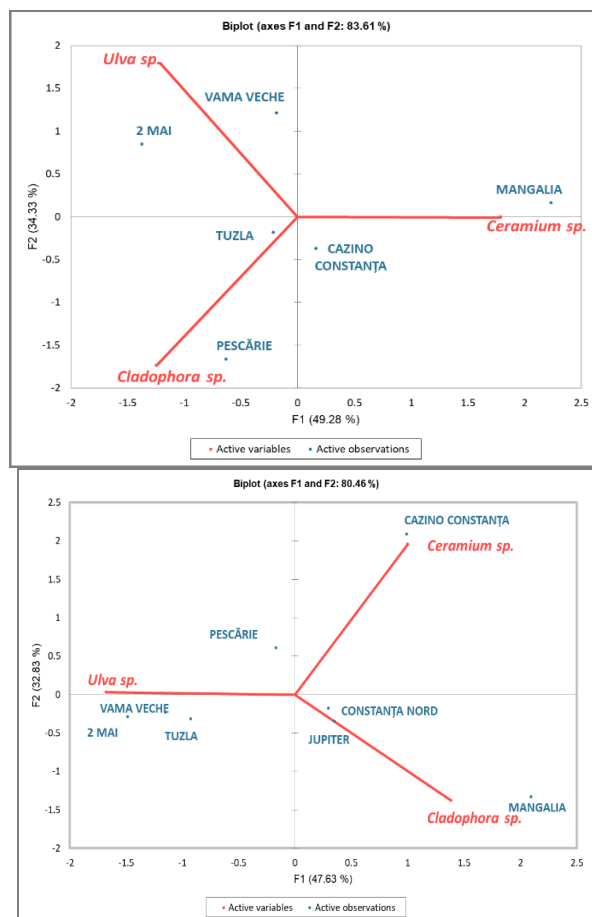


Figure 2. PCA (Principal Component Analysis) based on quantitative distribution of dominant opportunistic species during summer seasons 2020 (left) and 2021 (right)

During 2020 and 2021 summer seasons, within Upper infralittoral rock with variable annual green and red macroalgae habitat, the photophilic association *Ulva* – *Cladophora* – *Ceramium* was dominant, but the biomass proportion of the edifying species was different during these two summer seasons. Thus, in 2020 the main contributors were *Cladophora* species (66.58%), while in 2021, quantitatively dominant were *Ulva* species (95.62%) (Table 1).

Table 1. Opportunistic species SIMPER analysis – main contributors (2020 and 2021)

Summer season 2020					
Species	Average wet biomass (g/m ²)	Av. Sim	Sim/SD	Contrib%	Cum.%
<i>Cladophora</i> ssp.	1074.54	22.86	1.01	66.58	66.58
<i>Ulva</i> ssp.	669.69	10.40	0.79	30.29	96.87
Summer season 2021					
<i>Ulva</i> ssp.	1221.91	45.70	1.45	95.62	95.62

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Comparatively, analysing the quantitative distribution of the opportunistic *Ulva*, *Cladophora* and *Ceramium* species along Pescărie to Vama Veche, the clear dominance of chlorophytes at most sampling stations was observed in the past two years, except Mangalia (2020), where red algae of *Ceramium* genus dominated from quantitative point of view. However, in 2021, the quantitative dominance reverted to chlorophytes, especially to *Cladophora* species (Fig. 3).

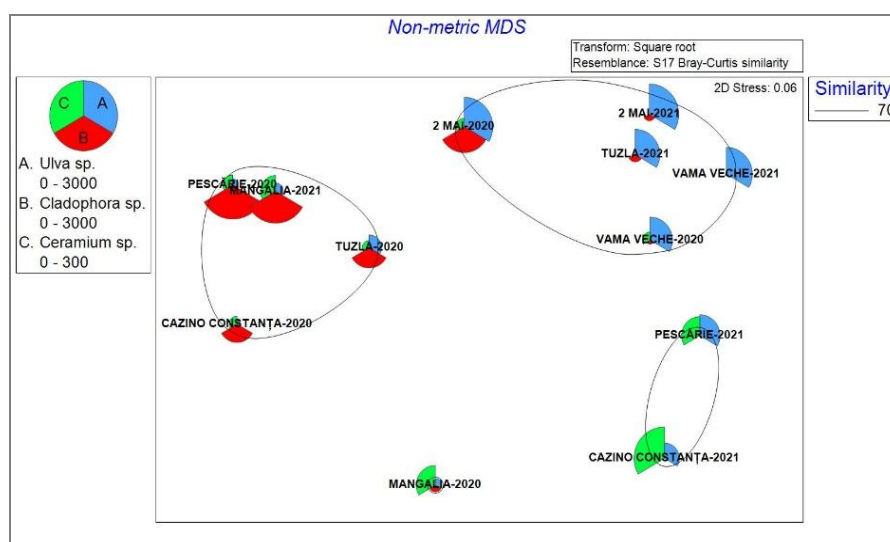


Figure 3. *n*-MDS (non-metric multi-dimensional scaling) for opportunistic dominant species (based on square root fresh biomass values)

In general, the phytobenthic associations qualitative and quantitative analysis within Upper infralittoral rock with variable annual green and red macroalgae and Upper-infralittoral rock dominated by *C. barbata* habitats showed a unitary, homogeneous structure, without major variations regarding the dynamics of the main species. Pescărie sampling station (where, in 2020, *Cladophora* ssp. experienced an intense development) and Cazino Constanța (where, in 2021, the perennial red alga, *Polysiphonia elongata* presented high biomass values) were the only stations that differentiated in this series of similarities (Fig. 4). At Mangalia, in the Upper infralittoral rock dominated by *C. barbata*, between 2 and 3 meters depth, the rare encrusted perennial red algae *Phymatolithon lenormandii* was observed.

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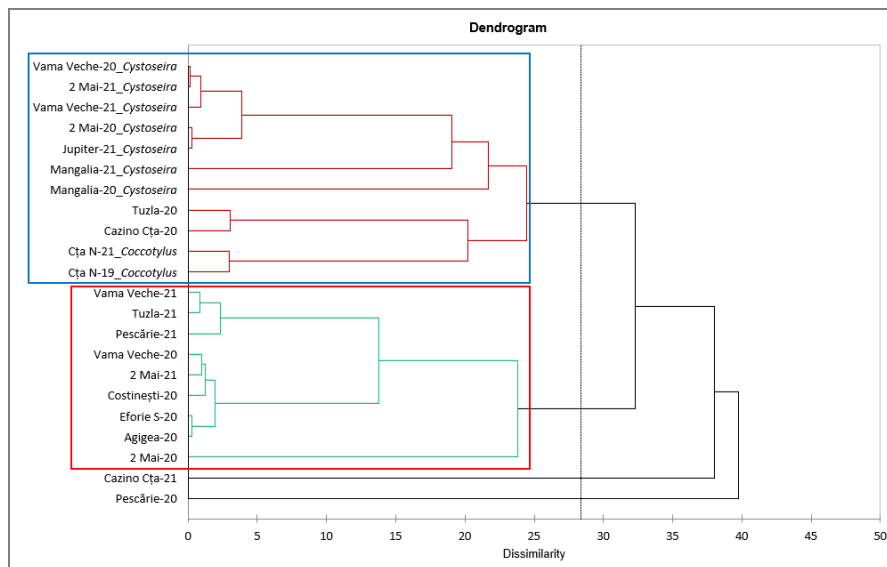


Figure 4. AHC (Agglomerative Hierarchical Clustering) for 2020 – 2021 sampling stations: sampling stations from Upper-infralittoral rock dominated by *C. barbata* and Upper infralittoral rock with *C. brodiei* (blue highlighted cluster); Upper infralittoral rock with variable annual green and red macroalgae sampling stations (red highlighted cluster)

The habitat-forming species *C. barbata* (Fig. 5) and *C. brodiei* (Fig. 5) have shown a favorable evolution along the Romanian Black Sea coast in the past two years, both in terms of quality and quantity. However, it should be considered that the special habitats, Upper infralittoral rock dominated by *C. barbata* and Upper infralittoral rock with *C. brodiei* have a punctiform distribution along the Romanian Black Sea shore (Fig. 6), with reduced distribution areas and are constantly exposed to anthropogenic pressures.



Figure 5. *C. barbata* (left) and *C. brodiei* (right) (Photo NIMRD)

These are unique habitats and require constant monitoring. Both characteristic species (*C. barbata* and *C. brodiei*) have a special ecological importance for the marine environment and are included in the Red List of endangered marine species along the Romanian coast (according to Ministerial Ord. 488/2020).

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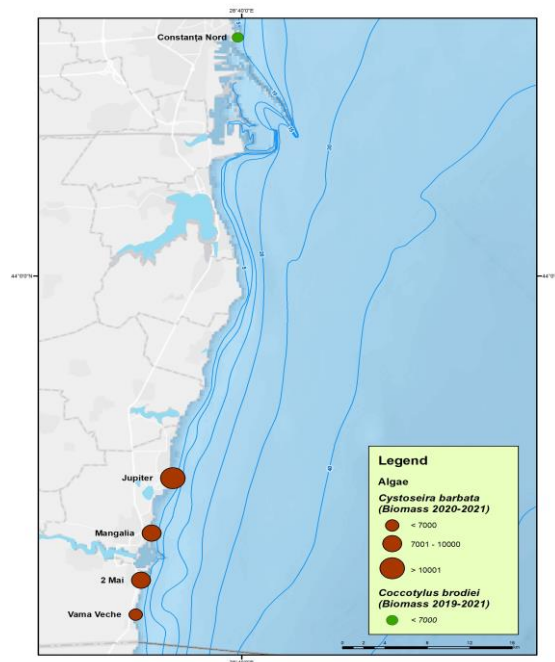


Figure 6. Wet biomass distribution of endangered species *C. barbata* and *C. brodiei* (2020-2021)

Between 2020 and 2021, the average biomass generated by the edifying species varied as follows:

- *C. barbata* – between 3,600 g/m² (Vama Veche, 2021) and over 14,000 g/m² (2 Mai, 2020) (Fig. 7). Also, a high biomass value (12,900 g/m²) was recorded in Jupiter in 2021.
- *C. brodiei* - between 700 g/m² (Constanța Nord, in 2021) and 1,500 g/m² (Constanța Nord in 2020).

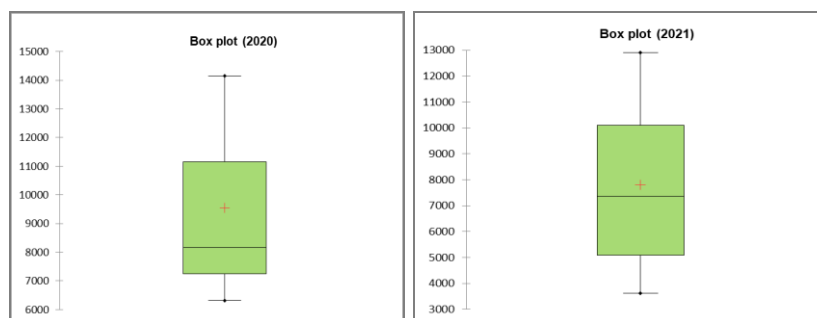


Figure 7. *C. barbata* – wet biomass variation between 2020 (left) and 2021 (right) summer seasons

Analyzing the last two years ecological status of the phytobenthic communities from Upper infralittoral rock with variable annual green and red macroalgae habitat, no major differences regarding the condition of algal flora were observed. A constant qualitative and quantitative evolution of the main algal components has been maintained, with a similarity of 70% among the sampling stations within the monitored hard substrate habitats (Fig. 8).

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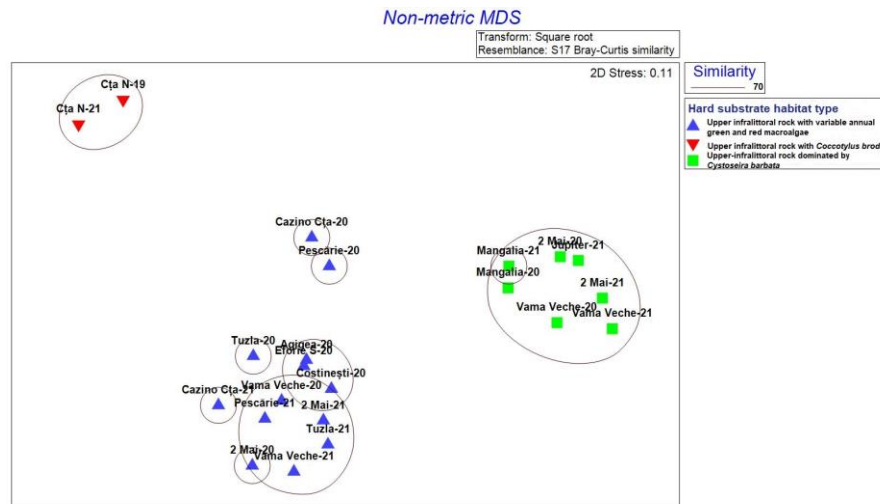


Figure 8. n-MDS (non-metric multi-dimensional scaling) indicating the similarity between sampling stations from hard substrate habitats (2020 – 2021)

Analyzing the ecological status of Upper infralittoral rock with variable annual green and red macroalgae habitat, it was observed that the good ecological status was not reached neither in 2020 nor in 2021. The EI values were below the threshold value (6) which indicates the achievement of the good ecological status. However, the two special habitats formed by perennial species *C. barbata* and *C. brodiei*, were found in good ecological status in the last two years (Fig. 9).

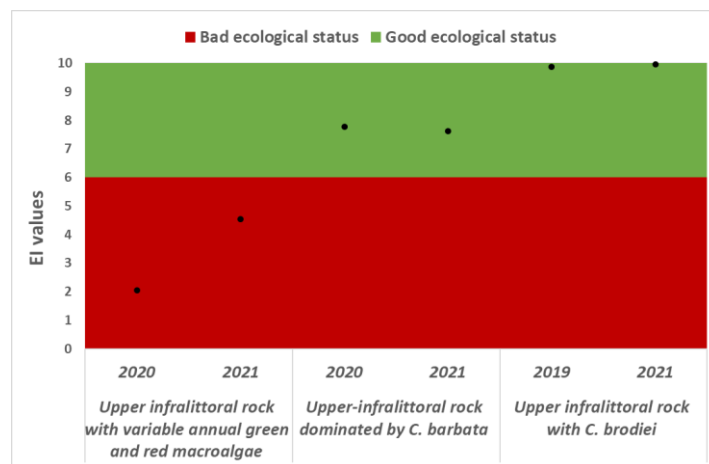


Figure 9. Ecological state evolution of the hard substrate habitats monitored during 2019 - 2021

3.2 Zoobenthic Communities From Marine Habitats With Hard Substrate

Following the sample processing, 65 macrozoobenthic species were identified (Annex 1), distributed per benthic invertebrate groups as follows: polychaete worms - 23 species (36%), bivalve molluscs - 5 (8%), gastropods - 6 species (9%), crustaceans - 22 species (34 %) and 8 species belonging to other groups such as nemertines, arachnids and insect larvae -13% (Fig. 10; Annex 1). The highest diversity was recorded at Tuzla/2m depth (42 species) and Eforie/6m (41 identified species). Also, a

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high number of species was recorded at Venus on 7 m depth and Tuzla/8 m depth, namely 33 and 36, respectively. A lower number of species was observed in Costinesti at 3 m and 10 m depths (between 21 and 24 species) (Annex 1). Bivalves *Mytilaster lineatus* and *Mytilus galloprovincialis* were the characteristic species on the hard substrate.

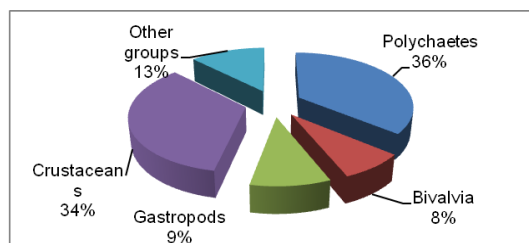


Figure 10. Percentages of zoobenthic groups based on species number

Irus irus (Fig. 11) and *Steromphala divaricata* (Fig. 11), two sensitive species included in the Red List of endangered marine species from the Romanian coast (according to Ministerial Ord. 488/2020) were also identified. The bivalve *I. irus*, an infralittoral rocky species, was found at Tuzla, Costinesti, Venus, on 2, 3, 7, 8 and 10 m depths, while the gastropod *S. divaricata*, a representative species for the infralittoral rock, was found only at Tuzla, on 8m depth.



Figure 11. *I. irus* (left) and *S. divaricata* (right) (Photo NIMRD)

Nonindigenous species *Rapana venosa* and *Anadara kagoshimensis*, already established in the infralittoral rocky habitats, were identified in 5 locations at different depths, in reduced number. The crustaceans had a constant presence, 5 of the total 22 identified species being frequently found at all depths: cirriped *Amphibalanus improvisus*, amphipods *Microdeutopus gryllotalpa*, *Monocorophium acherusicum*, *Hyale pontica*, *Plumulojassa ocia*. From decapoda (crabs) group, 4 species were identified: *Xantho poressa*, *Pilumnus hirtellus*, *Athanas nitescens* and *Pisidia longicornis*. The latter is mentioned in the Red List, as endangered species and was recorded at Eforie/6m, Venus/7m and Tuzla/8m. The highest average densities of the total macrozoobenthos were recorded at Costinesti and Mangalia on 2.5 and 3 m isobaths (over 90,000 ind/m²) and Eforie/6m (over 60,000 ind/m²). The lowest densities were recorded at Tuzla/8 m and Costinesti/10 m depth (up to 40,000 ind/m²). By taxonomic groups, the bivalves recorded the highest densities along all profiles (between 13,600 and 69,700 ind/m²), followed by crustaceans (between 833 and 48,117 ind /m²). The average densities recorded by the polychaetes varied between 400 ind/m² (Tuzla/2m) and 5,700 ind/m² (Mangalia/2.5m) (Fig. 12).

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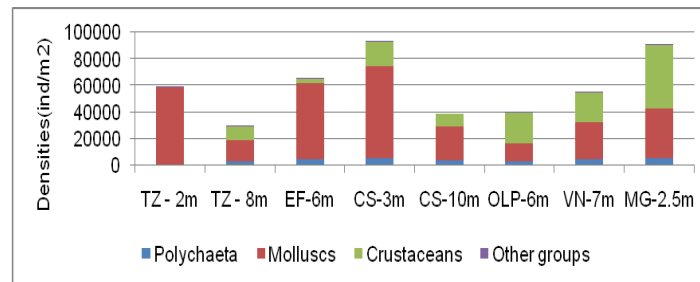


Figure 12. Average density variation of the main macrozoobenthic groups (2021)

Among bivalves, a significant contribution to the high-density values was due to *M. lineatus*, a mediolittoral characteristic species, which recorded the highest values (25,133 ex/m² - 66,000 ex/m²) on isobaths of 2m and 3m. With the depth increase, in the infralittoral rock habitats, *M. galloprovincialis* replaced *Mytilaster* populations, reaching the highest density values (18,500 ind/m²) at Costinesti/10m. Mixed colonies of *Mytilaster* and *Mytilus* were observed only at Tuzla/8 m. These two bivalves' populations (mostly juveniles) recorded similar density values (Fig. 13). The molluscs (especially *Mytilus* and *Mytilaster*) dominated in terms of biomass (over 20,000 g/m²). Other molluscs such as *S. divaricata* and *I. irus*, recorded lower biomasses (below 100 g/m²).

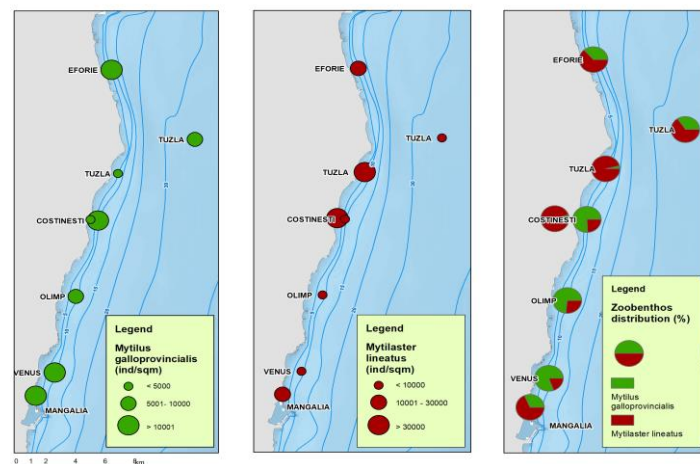
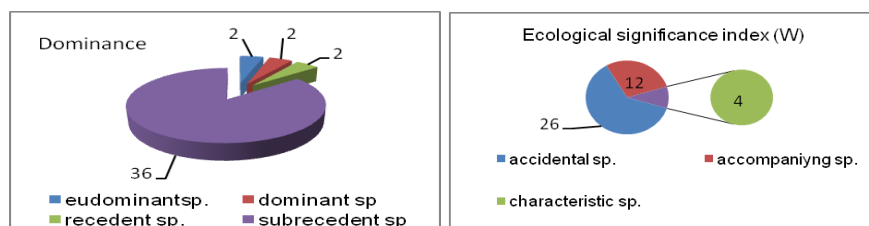


Figure 13. *M. galloprovincialis* and *M. lineatus* density distribution (2021)

Between 2 and 3 m depth, the synecological analysis performed based on the average density of the identified species (42 species), indicated 2 eudominant (*M. lineatus* and the amphipod *M. gryllootalpa*) and 2 dominant species (*M. galloprovincialis* and *Stenothoe monoculoides*). The ecological significance index (W) showed 26 accidental species, 12 accompanying species and 4 characteristic species (*M. lineatus*, *M. galloprovincialis*, *M. gryllootalpa* and *Lekanesphaera hookeri*) (Fig. 14).



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Figure 14. Synecological analysis (2 - 3 m depth)

Between 6 and 7 m depth, the synecological analysis indicated 3 eudominant (*M. lineatus*, *M. galloprovincialis* and *M. gryllotalpa*), and 2 subdominant species (*Platinereis dumerilli* and *A. improvises*). The ecological significance index (W) indicated the same three species as characteristic to infralittoral rocky habitat (Fig. 15).



Figure 15. Synecological analysis (6 - 7 m depth)

Between 8 and 10 m depth, the synecological analysis indicated 3 eudominant (*M. lineatus*, *M. galloprovincialis* and *A. improvisus*), 2 dominant (*Medicorophium runcicorne* and *M. gryllotalpa*), and 2 subdominant species (*P. dumerilli*, *Polydora cornuta*). The ecological significance index (W) showed the five eudominant and dominant species presented above, as characteristic to the infralittoral rocky habitats at indicated depths (Fig. 16).

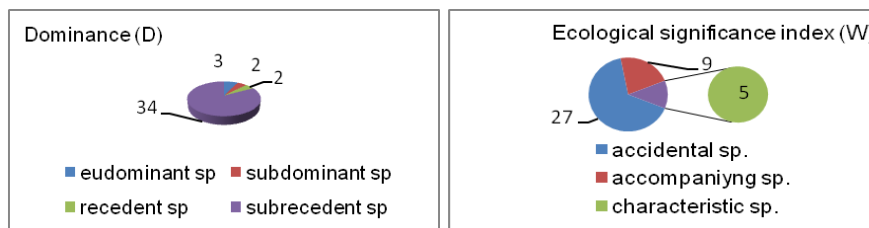


Figure 16. Synecological analysis (8 - 10 m depth)

4 CONCLUSIONS

The assessment of benthic communities from infralittoral rocky habitats in 2020 and 2021 led to the following conclusions:

- Organic pollution of the marine environment has led to the development of opportunistic species (both phytobenthic and zoobenthic).
- *M. galloprovincialis* and *M. lineatus* biocenosis, along with the dominance of the phytobenthic association *Ulva - Cladophora - Ceramium* are good example of the response of the marine environment to the intensification of anthropogenic pressures.
- During summer season, the main components of algal wrack deposits were *Ulva* ssp., *Cladophora* ssp. and *Ceramium* ssp.
- Green algae of the genera *Ulva* and *Cladophora*, compared to red algae, registered a more abundant development during summer season 2021 compared to 2020.
- Upper infralittoral rock with variable annual green and red macroalgae habitat was assessed in the past years as being in bad ecological status, while the

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special habitats, formed by *C. barbata* and *C. brodiei*, were found in good ecological status between 2019 and 2021.

- 65 macrozoobenthic species were identified in the analysed area. The highest diversity was recorded at Tuzla/2m and Eforie/6m.
- Five benthic species mentioned on the Red List of endangered marine species from the Romanian coast (the zoobenthic - *I. irus*, *S. divaricata* and *Pisidia longicornis* and the phytobenthic - *C. barbata* and *C. brodiei*) and two nonindigenous (*R. venosa* and *A. kagoshimensis*) were also found.
- The bivalve species *M. lineatus*, dominated between 2 and 3 m depth. At greater depths, the species *M. lineatus* was replaced by *M. galloprovincialis*, which recorded higher densities and biomass.
- According to the synecological analysis the bivalve species *M. lineatus* and *M. galloprovincialis* are eudominant and characteristic having a significant contribution in the ecosystem under functional aspect. Dominant species were the cirriped *A. improvisus*, amphipods *M. gryllotalpa* and *M. runcicorne*.
- Characteristic zoobenthic species on Infralittoral rock habitat were bivalve molluscs *M. lineatus* and *M. galloprovincialis*. Thus, the predominant biotope (habitat subtype) was Infralittoral rock dominated by Mytilids.

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Annex 1

The list of zoobenthic species identified at 2-10m depths on hard substrate (2021)

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No.	Species	2m	3m	6m	7m	8m	10m
1	<i>Leptoplana tremellaris</i>			*			
2	<i>Stylochus tauricus</i>			*		*	
3	<i>Leucocephalonemertes aurantiaca</i>			*			
4	<i>Amphiporus lactifloreus</i>			*	*		
5	<i>Pontolineus arenarius</i>				*		
6	<i>Tetrastemma melanocephalum</i>	*	*	*			
7	Nematoda			*			
8	<i>Platinereis dumerilii</i>	*	*	*	*	*	*
9	<i>Perinereis cultrifera</i>	*	*	*	*	*	
10	<i>Nereis zonata</i>	*	*	*	*	*	*
11	<i>Harmothoe imbricata</i>			*		*	
12	<i>Harmothoe impar</i>	*		*	*		*
13	<i>Prionospio cirrifera</i>					*	
14	<i>Prionospio multibranchiata</i>			*	*		
15	<i>Polydora cornuta</i>	*		*	*	*	*
16	<i>Genetyllis tuberculata</i>					*	
17	<i>Pygospio elegans</i>					*	
18	<i>Mysta picta</i>			*	*		
19	<i>Salvatoria clavata</i>	*	*	*	*	*	*
20	<i>Eulalia viridis</i>					*	
21	<i>Alitta succinea</i>	*		*	*	*	
22	<i>Hediste diversicolor</i>			*			
23	<i>Glycera tridactyla</i>					*	
24	<i>Exogone naidina</i>	*	*			*	
25	<i>Syllis gracilis</i>			*	*	*	
26	<i>Nereiphylla rubiginosa</i>	*		*		*	*
27	<i>Fabricia stellaris</i>	*				*	
28	<i>Heteromastus filiformis</i>	*					
29	<i>Capitella minima</i>	*	*	*	*	*	*
30	<i>Capitella capitata</i>	*		*		*	
31	<i>Lepidochitona caprearum</i>	*		*	*	*	
32	<i>Bittium reticulatum</i>	*		*	*	*	*
33	<i>Brachystomia scalaris</i>			*			*
34	<i>Tritia neritea</i>	*					
35	<i>Rissoa spendida</i>	*					
36	<i>Rapana venosa</i>	*	*		*	*	*
37	<i>Irus irus</i>	*	*		*	*	*
38	<i>Steromphala divaricata</i>					*	
39	<i>Anadara kagoshimensis</i>				*		*
40	<i>Mytilaster lineatus</i>	*	*	*	*	*	*
41	<i>Mytilus galloprovincialis</i>	*	*	*	*	*	*
42	<i>Amphibalanus improvisus</i>	*	*	*	*	*	*
43	<i>Microdeutopus gryllotalpa</i>	*	*	*	*	*	*
44	<i>Monocorophium acherusicum</i>	*	*	*	*	*	*
45	<i>Melita palmata</i>	*	*	*	*	*	
46	<i>Apherusa bispinosa</i>		*	*	*	*	
47	<i>Plumulojassa oca</i>	*	*	*	*	*	*
48	<i>Hyale pontica</i>	*	*	*	*	*	*
49	<i>Echinogammarus olivii</i>		*				
50	<i>Erichtonius difformis</i>	*		*			
51	<i>Stenothoe monoculoides</i>	*	*	*	*		
52	<i>Dexamine spinosa</i>	*		*	*		*
53	<i>Idotea balthica</i>	*					
54	<i>Lekanesphaera hookeri</i>	*	*	*			
55	<i>Dynamene bidentata</i>	*		*			
56	<i>Eurydice sp.</i>	*					
57	<i>Cumella limicola</i>	*		*		*	*
58	<i>Caprella acanthifera</i>	*					
59	<i>Phtisica marina</i>	*					
60	<i>Pisidia longicornis</i>			*	*	*	
61	<i>Athanas nitescens</i>					*	
61	<i>Xantho poresa</i>	*			*		
63	<i>Pilumnus hirtellus</i>	*			*		
64	<i>Rombognathus sp.</i>	*	*	*	*	*	
65	Chironomida larvae	*	*	*			