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ORIGINAL PAPERS

THE MACROZOOBENTHIC SPECIES OF THE INFRALITTORAL AND CIRCALITTORAL ZONE FROM THE ROMANIAN BLACK SEA COAST – A QUALITATIVE AND QUANTITATIVE ASSESSMENT

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Abstract. *The paper aims to present the results of the analysis of 20 macrozoobenthic samples collected in 2022 from the Romanian Black Sea shore. The depth of the sampling sites ranged between 12.3 m and 42 m, corresponding to the infralittoral and circalittoral zones. Qualitatively, 102 taxa of macrozoobenthos including 36 polychaetes, 25 crustaceans, 20 molluscs, 10 nemertians and 11 taxa representing other groups, were recorded. Polychaetes were predominantly abundant, representing 55.92% of density. Species such as Melinna palmata Grube, 1870, Heteromastus filiformis (Claparède, 1864) and Prionospio cirrifera Wirén, 1883 recorded the highest densities. Among crustaceans, the most abundant species were Ampelisca diadema (Costa, 1853), Phtisica marina Slabber, 1749 and Medicorophium runcicorne (Della Valle, 1893). The molluscs Abra prismatica (Montagu, 1808), Spisula subtruncata (da Costa, 1778) and Polititapes aureus (Gmelin, 1791) were considerably abundant as well. The average density of the benthic populations was 1571 indv/m^2 . By far the highest densities were recorded at two sites located close to the Danube's Sf. Gheorghe mouth, at depths of 12.3 m and 19.9 m, represented 18.40%, respectively 16.42% of the total average density. The other sites recorded densities not greater than 6%. The average biomass was 236.54 g/m^2 and was dominated by molluscs (90.74%). Among stations, Sf. Gheorghe (40.1 m) recorded the highest biomass, representing 42.60% of the total average biomass.*

Keywords: benthic fauna, distribution, Black Sea, polychaetes, Romania

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Introduction

The Black Sea forms a unique ecosystem due to its physico-chemical and biological characteristics. This basin is strongly influenced by freshwater inputs, and therefore nutrient loads [6]. In the last decades, the condition of zoobenthos from the

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Romanian Black Sea shelf was studied by several researchers, who have been able to follow closely, through monitoring surveys, the qualitative and quantitative changes that occurred within this component/ the macrozoobenthos distribution [1, 3, 4, 5, 8, 10].

The main threats identified on the Romanian sea coast, which can lead to the ecosystem degradation and loss of biodiversity, are represented by the growing urbanization in the shorelines, the growth of tourism and its related activities, destruction of the natural shoreline as a result of extensive urban development and pollution, a critical problem generated mainly by local industrial development [2].

Our study aims to investigate the macrozoobenthos diversity in the infralittoral and circalittoral zone, and to assess the benthic populations through analytical and synthetic ecological indices.For the references use: [1] or [2, 3, 4] or [2-4].

Material and Methods

For the present study, the macrobenthic samples were collected in July 2022 from the Romanian Black Sea coastal waters (Sf. Gheorghe to Vama Veche) (see Fig.1.). The depth of the sampling sites varied between 12.3 and 42 m. In total, 20 samples were collected by using a Van Veen grab (sampling area 0.1 m^2) on board of the research vessel „Steaua de Mare 1". The sampling was carried out in accordance with the agreed regional standards [11].

The collected grab samples were processed and stored onboard. The samples were photographed, sieved over a 0.5 mm mesh, stored in 5 L plastic buckets and preserved in a 4-10% formaldehyde saline solution for subsequent laboratory macrofaunal analysis.

In the laboratory, the samples were washed again through a 0.5 mm and 1 mm mesh, and were sorted under a stereomicroscope (OLYMPUS SZX10 - zoom range of 0.63x to 6.3x). All taxa were identified to the lowest possible taxonomic level (e.g., species) using specific identification keys and all names were updated according to the World Register of Marine Species (WoRMS) [13].

The quantitative analysis of zoobenthos consisted in assesing the number of organisms as density (indv/m^2) and biomass (g/m^2).

In order to identify the type of habitat, the sampling sites were transposed in GIS on the main types of broad habitats [12], then the result was compared with the field observations.

To reach the purpose of this study, a synecological analysis was carried out. Thus, analytical and synthetic ecological indices were analyzed using Microsoft Excel 365. Among the analytical ecological indices, we used the following:

Abundance (A) - the total number of a species individuals from a certain area; this parameter is usually expressed in indv/m^2. Depending on the value of this parameter, species are classified as rare, relatively common, abundant and very abundant [9].

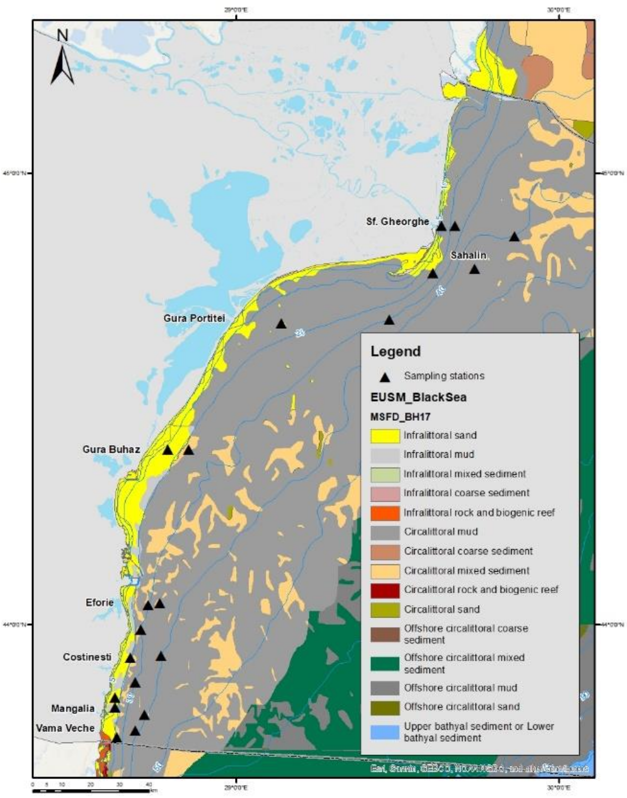

Fig. 1. Map of sampling sites

Dominance (D) (relative abundance) - the ratio between the numbers of specimens of a species and the sum of the total specimens of the other species in a studied area [9].This parameter is calculated with the formula: $D_A = (n_A / N) \times 100$, where D_A represents the dominance of species A, n_A- the number of individuals of species A in the sample and N represents the total number of individuals in the sample. According to the percentage, the species were grouped into the following dominance classes: D_1 – subrecedent species (< 1.1%), D_2 – recedent species (1.2 - 2%), D_3 – subdominant species (2.1 - 5%), D_4 – dominant species (5.1 – 10%) and D_5 – eudominant species (> 10.1%).

Constancy (Frequency) – C, F express the continuity of a species in a certain biotope [9]. This structural indicator represents the percentage ratio of the number of samples in which a certain species appears and the total number of analyzed samples. Constancy (C) is calculated with the formula $C_A = (n_A/ N) \times 100$, where C_A represents species A constancy, n_A – the number of samples of species A and N - the total number of samples.

According to the percentage, the species were grouped into the following constance classes: C_1 – accidental species (present in 1 – 25% of samples), C_2 –

accessory species (present in 25.1 – 50% of the samples), C_3 – constant species (present in 50.1 – 75% of the samples), and C_4 – euconstant species (present in 75.1 – 100% of the samples).

As a synthetic ecological index, Dzuba ecological significance index (W) was used, which reflects the relationship between the structural indicator (constancy) and the productivity indicator (dominance), showing the position of a species in a biocenosis. The formula of this index is: $W_A = (C_A \times D_A \times 100) / 10.000$, where: W_A = ecological significance of species A; C_A = species A constancy; D_A = dominance of species A [9].

According to the values of this index, the species were grouped into the following categories: W_1 – for values < 0.1% (subrecedent species), W_2 – for values between 0.1 – 1% (recedent species), W_3 – for values between 1.1 – 5% (subdominant species), W_4 – with values between 5.1 – 10% (dominant species); W_5 – with values > 10% (eudominant species). Class W_1 corresponds to accidental species, classes W_2 and W_3 correspond to accessory species and classes W_4 and W_5 correspond to characteristic species of the analyzed community [9].

The faunal data matrix was processed by univariate and multivariate methods of statistical analysis [7], using the software PRIMER®v.7. As a univariate analysis method, the diversity analysis was used, by which the following indices were calculated for each individual station:

The Margalef index (d) - represents the ratio between the number of species in a certain biotope and the logarithm of the number of recorded specimens. This index is calculated with the formula: $D = (S - 1) / \log_e N$, where: S - the number of species in the analyzed biotope or biocenosis, N - the total number of specimens recorded in the analyzed biotope or biocenosis.

Shannon – Wiener diversity index is calculated with the formula $H' = - \Sigma P_i \log P_i$, where: H' - the Shannon – Wiener diversity index, P_i - the proportion of individuals by which species i is represented in the community, $P_i = n/N$, where n- the number of individuals of species i and N = the number total individuals of all species in the sample.

Pielou's evenness index (J' or E) reflects the degree of balance or imbalance of a biocenosis, taking into account the fact that a biocenosis with maximum diversity has maximum stability; $J' = H' / \log_e S$, where: H'- Shannon – Wiener diversity and S = total number of species in the sample.

PRIMER (Clarke & Gorley, 2015) was used to carry out multivariate analyses on the faunal data [7]. All species abundance matrix was fourth root transformed. The fourth root transformed data matrix was used to prepare a Bray-Curtis similarity matrix before the classification and cluster analysis. The cluster analysis aimed at finding "natural groupings" of samples, i.e. samples within a group that are more similar to each other, than they are similar to samples in different groups. The result was represented graphically in a dendrogram.

Results and Discussions

From the total sampling sites, five sites correspond to the infralittoral zone while 15 sites to the circalittoral zone. The sampling sites covered the following habitat types: infralittoral sands, infralittoral muds, circalittoral muds and circalittoral mixed sediments [12].

Qualitative and quantitative macrofauna investigations

Through the analysis of the 20 samples, 102 taxa were identified (see Table 1.), belonging to the following groups: Polychaeta (55.92%), Crustacea (22.98%), Mollusca (16.96%), Nemertea (2.36%), Anthozoa (0.99%) and “Other groups” which are represented by taxonomic groups with low densities (oligochaetes, phoronids, echinoderms,tunicates and pycnogonids.

For the study area, was obtained an average density of 1571 indvd/m^2 and an average biomass of 236.54 g/m^2.

Regarding the estimated faunal density, the highest values were recorded at Sf. Gheorghe site, at the depth of 12.3 m (5780 indvd/m^2) and at Sahalin site, at the depth of 19.9 m (5160 indvd/m^2) representing 18.40% and 16.42% respectively, of the total density. The lowest densities were recorded at Neptun (33.2 m), Gura Portiței (15 m) and Gura Buhaz (13.9 m), representing 2.13%, 2.16% and 2.20% of the total density.

In terms of biomass, the major contributors are represented by mollusks, which constitutes 91% of the total estimated biomass. At Sf. Gheorghe station (40.1 m) was registered the highest biomass, representing 42.60% of the total biomass, followed by Tuzla (31.9 m) - 12.99% and Vama Veche at 23.1 m depth (9.17%). The lowest biomass percentages were recorded at Eforie Nord (35.5 m) - 0.01%, Neptun (33.2 m) - 0.02% and Gura Buhaz (13.9 m)- 0.27%.

Table 4. List of macrozoobenthic taxa identified in the analyzed stations/the infralittoral and circalittoral zone in July 2022

Crt. no.	Taxa
1	*Halichondria (Halichondria) panicea* (Pallas, 1766)
2	*Diadumene lineata* (Verrill, 1869)
3	*Sagartiogeton undatus* (Müller, 1778)
4	*Amphiporus bioculatus* McIntosh, 1874
5	*Carinina heterosoma* Müller, 1965
6	*Cyanophthalma obscura* (Schultze, 1851)
7	*Leptoplana tremellaris* (Müller OF, 1773)
8	*Leucocephalonemertes aurantiaca* (Grube, 1855)
9	*Micrura fasciolata* Ehrenberg, 1828
10	Nemertea
11	*Pontolineus sp.*

Crt. no.	Taxa
12	*Prostomatella sp.*
13	*Tetrastemma sp.*
14	Oligochaeta
15	*Alitta succinea* (Leuckart, 1847)
16	*Amphicorina armandi* (Claparède, 1864)
17	*Aonides paucibranchiata* Southern, 1914
18	*Aricidea sp.*
19	*Capitella capitata* (Fabricius, 1780)
20	*Capitella minima Langerhans, 1880*
21	*Eulalia sp.*
22	*Eulalia viridis* (Linnaeus, 1767)
23	*Fabricia sabella* (Ehrenberg, 1836)
24	*Genetyllis tuberculata* (Bobretzky, 1868)
25	*Harmothoe imbricata* (Linnaeus, 1767)
26	*Harmothoe reticulata* (Claparède, 1870)
27	*Hediste diversicolor* (O.F. Müller, 1776)
28	*Heteromastus filiformis* (Claparède, 1864)
29	*Lagis koreni* Malmgren, 1866
30	*Leiochone leiopygos* (Grube, 1860)
31	*Melinna palmata* Grube, 1870
32	*Micronephthys longicornis* (Perejaslavtseva, 1891)
33	*Nephtys cirrosa* Ehlers, 1868
34	*Nephtys hombergii* Savigny, 1818
35	*Phyllodoce maculata* (Linnaeus, 1767)
36	*Platynereis dumerilii* (Audouin & M.- Edwards, 1833)
37	Polychaeta
38	*Polycirrus sp.*
39	*Polydora cornuta* Bosc, 1802
40	*Prionospio cirrifera* Wirén, 1883
41	*Prionospio multibranchiata* Berkeley, 1927
42	*Pygospio elegans* Claparede, 1863
43	*Scolelepis squamata* (Müller, 1806)
44	*Sphaerosyllis bulbosa* Southern, 1914
45	*Spio decorata* Bobretzky, 1870
46	Spionidae
47	*Spirobranchus triqueter* (Linnaeus, 1758)
48	*Streblospio shrubsolii* (Buchanan, 1890)

Crt. no.	Taxa
49	*Syllis gracilis* Grube, 1840
50	*Terebellides stroemii* M. Sars, 1835
51	*Phoronis euxinicola* Selys-Longchamps, 1907
52	*Conopeum seurati* (Canu, 1928)
53	*Cryptosula pallasiana* (Moll, 1803)
54	*Abra alba* (W. Wood, 1802)
55	*Abra prismatica* (Montagu, 1808)
56	*Acanthocardia paucicostata* (G. B. Sowerby II, 1834)
57	*Anadara kagoshimensis* (Tokunaga, 1906)
58	*Calyptraea chinensis* (Linnaeus, 1758)
59	*Chamelea gallina* (Linnaeus, 1758)
60	*Gouldia minima* (Montagu, 1803)
61	*Lepidochitona caprearum* (Scacchi, 1836)
62	*Modiolus adriaticus* Lamarck, 1819
63	*Mya arenaria* Linnaeus, 1758
64	*Mytilaster lineatus* (Gmelin, 1791)
65	*Mytilus galloprovincialis* Lamarck, 1819
66	*Parvicardium exiguum* (Gmelin, 1791)
67	*Parvicardium simile* (Milaschewitsch, 1909)
68	*Pitar rudis* (Poli, 1795)
69	*Polititapes aureus* (Gmelin, 1791)
70	*Retusa truncatula* (Bruguière, 1792)
71	*Spisula subtruncata* (da Costa, 1778)
72	*Steromphala divaricata* (Linnaeus, 1758)
73	*Tritia reticulata* (Linnaeus, 1758)
74	*Callipallene phantoma* (Dohrn, 1881)
75	*Ampelisca diadema* (Costa, 1853)
76	*Amphibalanus improvisus* (Darwin, 1854)
77	Amphipoda
78	*Apherusa bispinosa* (Spence Bate, 1857)
79	*Apseudopsis ostroumovi* Bacescu & Carausu, 1947
80	*Cardiophilus baeri* G.O. Sars, 1896
81	*Crangon crangon* (Linnaeus, 1758)
82	*Deflexilodes gibbosus* (Chevreux, 1888)
83	*Diogenes pugilator* (Roux, 1829)
84	*Iphinoe elisae* Băcescu, 1950
85	*Iphinoe maeotica* Sowinskyi, 1893

Crt. no.	**Taxa**
86	*Medicorophium runcicorne* (Della Valle, 1893)
87	*Melita palmata* (Montagu, 1804)
88	*Microdeutopus anomalus* (Rathke, 1843)
89	*Microdeutopus damnoniensis* (Bate, 1856)
90	*Microdeutopus gryllotalpa* A. Costa, 1853
91	*Microdeutopus versiculatus* (Bate, 1856)
92	Mysida
93	*Nototropis guttatus* Costa, 1853
94	*Paramysis (Longidentia) kroyeri* (Czerniavsky, 1882)
95	*Perioculodes longimanus longimanus* (Bate & Westwood, 1868)
96	*Phtisica marina* Slabber, 1749
	Synchelidium maculatum Stebbing, 1906
98	*Upogebia pusilla* (Petagna, 1792)
99	*Xantho poressa* (Olivi, 1792)
100	*Leptosynapta inhaerens* (O.F. Müller, 1776)
101	*Amphiura stepanovi* Chernyavskii, 1861
102	*Ascidiella aspersa* (Müller, 1776)

The analysis of zoobenthos samples from the infralittoral zone revealed 54 taxa which, in terms of their frequency, were grouped into 6 euconstant species, 3 constant species, 16 accessory species and 29 accidental species.

The euconstant species in the infralittoral zone are *Prionospio cirrifera* Wirén, 1883, *Micronephthys longicornis* (Perejaslavtseva, 1891), *Ampelisca diadema* (Costa, 1853), *Heteromastus filiformis* (Claparède, 1864), *Capitella capitata* (Fabricius, 1780) and *Alitta succinea* (Leuckart, 1847).

From the same littoral zone, the constant species are *Polydora cornuta* Bosc, 1802, *Crangon crangon* (Linnaeus, 1758) and *Conopeum seurati* (Canu, 1928).

The euconstant species in the infralittoral zone are *Prionospio cirrifera* Wirén, 1883, *Micronephthys longicornis* (Perejaslavtseva, 1891), *Ampelisca diadema* (Costa, 1853), *Heteromastus filiformis* (Claparède, 1864), *Capitella capitata* (Fabricius, 1780) and *Alitta succinea* (Leuckart, 1847). From the same littoral zone, the constant species are *Polydora cornuta* Bosc, 1802, *Crangon crangon* (Linnaeus, 1758) and *Conopeum seurati* (Canu, 1928).

For the Ecological significance index calculation (W-Dzuba index) in the infralittoral zone, were eliminated three species for which we do not have density values but were used in this study for their importance in the qualitative analysis. Thus, were identified two eudominant species, two dominant species, four subdominant species, 15 recedent species and 28 subrecedent species.

The eudominant species identified in the infralittoral sampling sites are *Ampelisca diadema* (Costa, 1853) and Heteromastus filiformis (Claparède, 1864), while the dominant species are *Prionospio cirrifera* Wirén, 1883 and *Micronephthys longicornis* (Perejaslavtseva, 1891).

A number of 85 taxa were identified in the circalittoral zone which, in terms of their frequency were grouped into three euconstant species, 10 constant species, 18 accessory species and 54 accidental species. The euconstant species are *Nephtys hombergii* Savigny, 1818, *Heteromastus filiformis* (Claparède, 1864) and *Capitella capitata* (Fabricius, 1780).

From the same littoral zone, the constant species with the highest percentage are *Medicorophium runcicorne* (Della Valle, 1893), *Harmothoe reticulata* (Claparède, 1870), *Ampelisca diadema* (Costa, 1853) and *Terebellides stroemii* M. Sars, 1835,

Through the Ecological significance index (W- Dzuba index) 12 subdominant species, 19 recedent species and 54 subrecedent species were identified in the circalittoral zone. The subdominant species with the highest percentage are *Harmothoe reticulata* (Claparède, 1870), *Nephtys cirrosa* Ehlers, 1868, *Medicorophium runcicorne* (Della Valle, 1893) and *Capitella capitata* (Fabricius, 1780).

The amphipod *Ampelisca diadema* was the most abundant species in the infralittoral zone, as well as the eudominant species with the highest percentage. In the circalittoral zone, the polychaete *Melinna palmata* recorded the highest abundance value, being a eudominant species, also.

The diversity analysis highlights 2 sampling sites, which recorded the highest values in terms of biodiversity. At Costinești (42 m) and Saturn (12.7 m), the Margalef index recorded values of 4.11 and 4.02 respectively, the Shannon-Wiener index recorded values of 2.86 and 2.80 respectively, these indicating an increased fauna diversity.

A low diversity of benthic fauna is recorded at Sf. Gheorghe (12.3 m), Gura Portiței (32.3 m) and Gura Buhaz (13.9 m), indicated by values below 2.0 of the Margalef index.

Pielou's evenness index varies between 0.39-0.88, approximately directly proportional to the Shannon-Wiener diversity index, which proves that the numerical abundance of the identified species is not equitably distributed among them, and the community does not reach the highest level of stability.

The dendrogram produced by CLUSTER analysis is a complex representation of the relationship between the 20 macrozoobenthic samples (see Fig. 2.). The SIMPROF test identified 5 main groups that can be interpreted as distinct faunal assemblages.

Group d, consisting of a single sampling site (Sf. Gheorghe 40.1 m), was excluded from the similarity analysis, due to the significant difference in the faunal composition, compared to the other stations.

Group b - the similarity was 42.37%. Seven species accounted for over 72% of the total within-group similarity. The species with the highest contribution were *Ampelisca* *diadema* (13.39%), *Micronephthys longicornis* (18.35%) and *Capitella capitata* (12.03%).

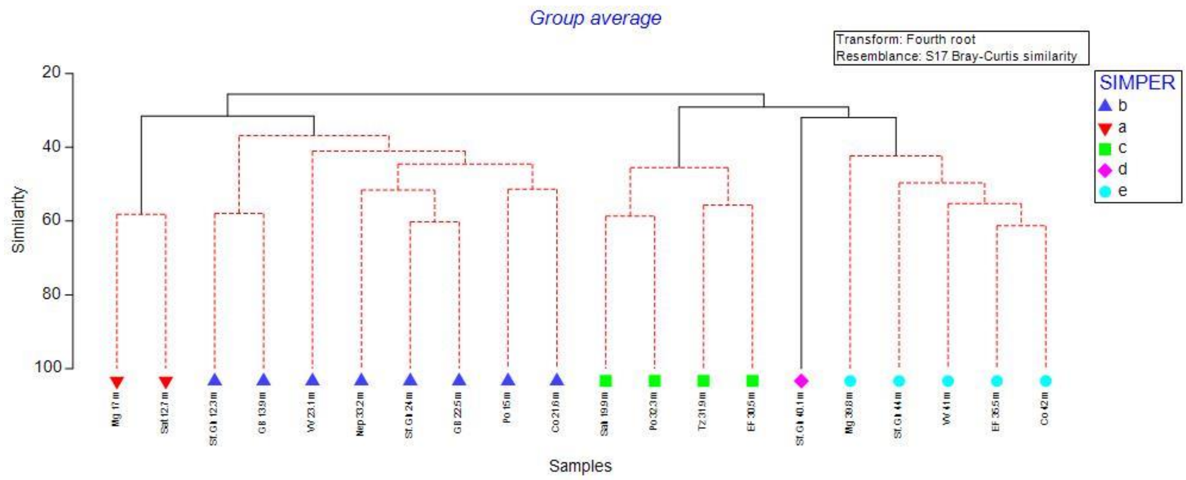

Fig. 2. Dendrogram produced from Cluster analysis with SIMPROF test in Primer

Group a – the similarity was 58.20%. Nine species accounted for over 74% of the total within-group similarity. The species with the highest contribution were *Prionospio cirrifera* (11.63%), *Microdeutopus gryllotalpa* (10.60%) and *Syllis gracilis* (9.38%).

Group c – the similarity was 49.41%. Seven species accounted for over 71% of the total within-group similarity. The species with the highest contribution were *Abra prismatica* (14.42%), *Acanthocardia paucicostata* (10.37%) and *Polititapes aureus* (9.99%).

Group e – the similarity was 48.99%. Ten species accounted for over 72% of the total within-group similarity. The species with the highest contribution were *Terebellides stroemii* (14.15%), *Nephtys hombergii* (11.56%) and *Heteromastus filiformis* (8.87%).

Regarding the dissimilarity between groups, the highest percentage was registered between *groups a and c* (83.75%). A number of 29 species accounted for 69% of the total within-groups disimilarity. This incresead dissimilarity can be explained by the significant difference in habitats and therefore fauna, for each group: stations belonging to *group a* were distributed on infralittoral sands while the stations belonging to *group c* were distributed on circalittoral muds and mixed circalittoral sediments.

Conclusions

(1) The study area comprised the infralittoral and the shallow circalittoral zone from the Romanian Black Sea shore and is characterised by infralittoral muds, infralittoral sands, circalittoral muds and mixed circalittoral sediments.

(2) 102 taxa including 36 polychaetes, 25 crustaceans, 20 molluscs, 10 nemertians and 11 taxa representing other groups, were identified.

(3) The most dominant benthic group were polychaetes, which represented 55.92% of the faunal density. For the entire studied area, were obtained an average density of 1571 indvd/m^2 . The average biomass was 236.54 g/m2, being dominated by 90.74% of molluscs.

(4) The amphipod *Ampelisca diadema* was the most abundant species in the infralittoral, as well as the eudominant species with the highest percentage. In the circalittoral, the eudominant species *Melinna palmata* recorded the highest abundance value.

(5) Both the Shannon-Wiener index and the Margalef index recorded the highest values at Costinești (42 m) and Saturn (12.7 m), indicating a high local faunal diversity.

(6) The multivariate analyzes carried out in PRIMER allowed the identification of 5 distinct benthic communities. Taxa with the highest contribution were identified using SIMPER analysis.

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