ROMANIAN ACADEMY
INSTITUTE OF BIOLOGY - BUCHAREST

SUMMARY OF THE PhD THESIS

Contributions to the integrated biological knowledge of macrophytic vegetation in coastal waters along the Romanian Black Sea coast

Scientific Coordinators:

ACADEMICIAN Marian-Traian GOMOIU
INSTITUTE OF BIOLOGY - BUCHAREST

ACADEMICIAN Octavian POPESCU
INSTITUTE OF BIOLOGY - BUCHAREST

PhD Student
DUMITRESCU (MARIN) Oana Alina

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CONTENT

INTRODUCTION

The macrophytic vegetation (phytobenthos) is a major component of primary producers, the basis of the existence and development of life in the marine environment, a true underwater engine of material and energy flows. This component includes the macroalgae (Chlorophyta, Ocrophyta, Rodophyta) and the marine phanerogams (Tracheophyta). The phytobenthic communities ecological value is inestimable, simply because it represent an important part in the food chain, a space for feeding, defense and reproduction for zoobenthic communities and fish. The macrophytes have an important role in stabilizing the substrate, preventing coastal erosion, a worrying phenomenon in the past years. The macroalgae are an unique domain able to maintain the biological balance in the marine environment, with an important ecological value doubled by their economic importance. Many species offer possibilities for exploration in most diverse domains (pharmaceutical, cosmetic, industrial, etc.), their own active principles might be an answer to certain society problems in the future. Both macroalgae and marine phanerogams are constantly threatened by population degradation phenomena, species decline or even extinction, due to the fact that they are located in the coastal area, an area under permanent anthropogenic influence.

For the Romanian Black Sea coast, few studies exists in the last decades in relation with biological and ecological aspect of macrophytic vegetation, a very important part that needs to be fully known before a potential economic valorification. Taking into account the importance of macrophytic submerged vegetation, a detailed knowledge of the current condition for this environmental component was required. The thesis consists in 243 pages and contributes to scientific database update with new information on the state of the phytobenthic communities in the context of permanent changes in the coastal zone, as a result of increasing anthropogenic activities. Personal contributions cover a period of 11 years (2009 - 2019), and during this period five major directions, which define the current condition of phytobenthic communities, have emerged:

1) the pronounced uniformity of phytobenthic associations.
2) the low identified number of perennial species compared to previous decades.
3) the clear dominance of green algae during the warm season.
4) the reappearence of some considered extinct species
5) a slightly regeneration process for the key ecological role species

The thesis includes 156 figures, 32 tables (all original), over 150 original photos, a bibliographic list of 109 references and one annex. The thesis begins with a first theoretical part, which refers to the current state of knowledge, briefly presenting the history of phytobenthic research in the Black Sea and a critical analysis of the current state of knowledge of submerged vegetation along the Romanian Black Sea coast from early XX century till present. This part includes 21 pages. The second part contains personal contributions, developed on 222 pages.

The aim of this thesis is to provide recent data regarding the specific diversity and species distribution, distribution area of key ecological value perennial species, quantitative assessment of macrophytes and phanerogams and trend evolution of phytobenthic species along the Romanian coast, all information obtained as a result of personal research. The thesis is completed with a detailed species list identified along the Romanian Black Sea coast from 1930 until present, based on a thorough bibliographic synthesis and personal data. The specific objectives of the thesis, achievable through a constant and dedicated monitoring program and laboratory experiments, are the following:

1) establishing the current qualitative composition of the submerged vegetation from the Romanian Black Sea coast.
2) elaboration of the phytobenthic species list from the last century.
3) analysis of the current ecological status compared to past decades situation.
4) analysis of the quantitative distribution of submerged vegetation along the Romanian coast during the last decade.
5) establishing the influence of recent coastal anthropogenic constructions on phytobenthic communities.
6) analysis of the ecological state of the coastal marine environment through phytobenthic elements.
7) performing macroalgal cultures under laboratory controlled conditions throught reproductive elements manipulation.

1. PHYTOBENTHIC RESEARCH EVOLUTION

The first chapter presents a short history of phytobenthic research in the whole Black Sea, with direct reference to the Romanian Black Sea coast **(subchapter 1.1),** along with a comprehensive critical analysis of the current state of knowledge of submerged vegetation along the Romanian Black Sea coast **(subchapter 1.2).** The macrophytes study began more than a century ago and was approached by various specialists, facilitated by the establishment of the Zoological Research Station in Agigea in 1926 and of the Biooceanographic Constanta Institute in 1932. The specialists did not limit themselves to a simple qualitative analysis of this ecosystem component, but deepened this varied domain, from interspecific populations relationships, to seasonal distribution and even valorification possibilities of this resource.

The thesis presents a detailed analysis of all the research stages from the beginning of the 20th century, until present. The studies began with field observations and descriptions of morphological characteristics for green, red and brown algae, followed by quantitative assessments and a description of the relationship between macroalgae and ecological factors. For the Romanian Black Sea coast, the macrophytes studies began in 1907, along with the research of Emanoil Teodorescu, "Materiaux pour la flore algologique de la Mer Noire", in which the author presents a first list of macrophytes particularly for the Romanian coast, along with morphological descriptions (Porumb, 1999 - 2000).

The interwar period and the one after the Second World War are dominated by the systematic and descriptive approach of the great algologist Maria Celan (Celan, 1935, Celan, 1940). Since the 40s, Maria Celan approaches a completely new aspect for the study of macroalgal communities, namely the valorification possibility of algal biomass, the researcher proving once again her visionary qualities (Celan, 1943). From the mid-50s to early 70s, studies are diversifying, approaches are becoming more complex and collaborations between specialists are emerging. Phytobenthic component research is dominated by Maria Celan, but there are new researchers, such as Hilarius Skolka, Adrian Bavaru or Maria Stadniciuc that distinguish. New research areas appear, such as identification and description of new species for the Romanian coast (Celan, 1962 b), embryology and cytology approach (Celan, Bavaru, 1968) or the influence of seasonal succession on phytobenthic communities (Celan *et al.,* 1969).

Early 1970s to the mid-1980s period is the one in which submerged vegetation studies experienced the greatest diversification, being considered the most complex period regarding research directions, from detailed articles on reproduction periods and embryonic stages of species (Bavaru, 1971), up to quantitative evaluations of species with exploitable potential (Vasiliu, Bodeanu, 1972). Since the 1990s, scientific articles became reduced, but in the same time the research addressed areas are diversifying, from synthesis papers to radioecology, eutrophication and active principles. Nowadays, the approaches are more and more complex, but the conclusion of Maria Celan, formulated since the 60s, namely that the submerged vegetation suffered an accentuated qualitative decline along the Romanian Black Sea coast, remains valid.

2. MATERIAL AND METHODS

The chapter begins with a presentation of the sampling method, qualitative and quantitative analysis of phytobenthic samples along the riparian countries **(subchapter 2.1),** followed by the presentation of the current monitoring program addressed in the past 11 years **(subchapter 2.2),** a program which allowed a constant monitoring of the submerged vegetation, with all its changes (abundant development of macroalgae during the summer season, identification of new species, revision of the Red List endangered species, etc.). The sampling monitoring network was initially established after literature review and field expeditions in order to concretely observe the local conditions. In support of this approach, 70s (Bavaru, 1972), 80s (Vasiliu, 1984), and later 90s studies (Sava, 2000) provided basic information regarding the sampling stations and dominant species from that periods.

Between 2009 and 2019, the samples were collected along the coastal strip Năvodari - Vama Veche, from the following stations considered representative for the current macroalgal associations: Năvodari, Mamaia, Pescărie, Constanța Nord, Cazino Constanța, Agigea, Eforie Nord, Eforie Sud, Tuzla, Costinești, Saturn, Mangalia, 2 Mai și Vama Veche (Fig.1). In the past years, the effort research has focused on the extreme southern area, namely the coastal strip

Mangalia - 2 Mai - Vama Veche (Fig.1), due to the presence of some endangered, extremely sensitive to anthropogenic disturbances species, which requires a constant monitoring.

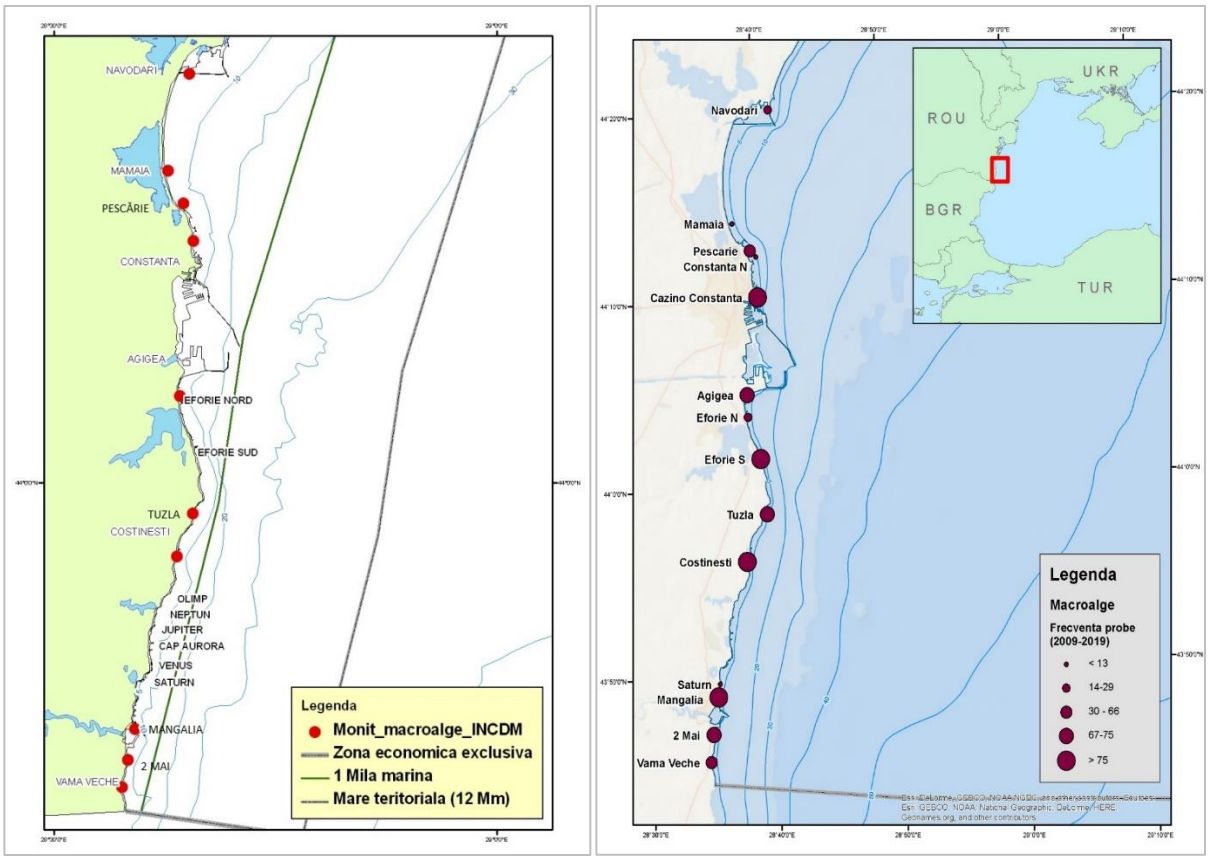


Fig.1. Sampling stations map (left) and frequency of sampling (right) between 2009 and 2019

The samples were collected each year during warm season (between May and September-October), the period of maximum development for phytobenthos along the Romanian Black Sea coast. 3 replicates were collected from each depth range (generally between 0 to 3 meters, except for Năvodari, with samples between 0 to 5 meters, Mamaia, with samples between 1 to 5 m and Constanța Nord with samples between 0 to 8 m depth), with a metal frame of 20x20 cm, in accordance with the so-called "square method". The samples were collected form hard substrate (for sampling macroalgae) and sandy (for sampling marine phanerogams). In case of endangered species (e.g. *Cystoseira*, *Coccotylus*, *Zostera*) only one sample was collected from each depth gradient, in order not to damage the algal material. A total number of 789 samples was collected, further analyzed from a qualitative (cells and thallus measurements, epiphytes observations, identification of reproductive elements) and quantitative (calculation of total wet biomass) point of view.

The qualitative analysis was performed on fresh biological material. Some long-term conservation methods, for additional analyzes or subsequent confirmations of rare species, also exist. Keeping them in preservative solutions (with formalin 4% or alcohol with a concentration

higher than 40^0), is one of the methods, the disadvantage being that after a certain period of time these solutions lead to cells depigmentation and thus some specific analyzes can be compromised. The most widely used method of preserving algae is considered to be the herbarium, kept in special environmental conditions.

The analysis of physico-chemical and bio-chemical parameters was performed by colleagues from the Laboratory of Physico-Chemical Measurements and Analyzes within NIMRD "Grigore Antipa".

Another approach of this thesis were the macroalgae cultures. The experimental cultures were performed in NIMRD "Grigore Antipa" laboratories, through reproductive elements (spores) manipulation. Two macroalgal species were selected – the stenothermic red algae, *Porphyra leucosticta* and the perennial brown alga, *Cystoseira barbata* - to be grown under laboratory controlled conditions, their selection being closely related to the provided ecosystem services. Thus, *P. leucosticta* is a species with a remarkable antioxidant activity (Mayalen *et al*., 2009), large amount of vitamin C, carotenoids and chlorophyll *a*, and its cultivation under laboratory controlled conditions can provide algal material for a future exploitation. In the same time, *C. barbata* is a species that distinguished by its ecological value, included in the Red List of endangered marine species, it's experimental cultivation providing important information on its reproductive process and intermediate stages of development. *C. barbata* laboratory culture provides algal material for future ecological reconstruction activities.

Characteristic ecological indices were applied in order to define the ecological status of the coastal zone, in accordance with the requirements of the two European Directives - the Water Framework Directive and the Marine Strategy Framework Directive. The data were processed by statistical methods, using PRIMER 7 program (v.7.0.17), and for a proper graphical representation of the seasonal variation of algal biomass, Ocean Data View v. 4.5.3 and ArcGIS 10.5 were used.

3. RESULTS AND DISCUSSIONS

3.1. SUBMERGED MACROPHYTIC VEGETATION CHARACTERIZATION FROM COASTAL WATERS ALONG THE ROMANIAN BLACK SEA COAST

3.1.1. Qualitative analysis of macrophytic vegetation from the Romanian Black Sea coast

In this chapter, the qualitative variation of phytobenthic populations was analyzed, from highlighting the variability of species number over the decades, to the detailed presentation of the morphological features of the main phytobenthic species identified during the study period. The chapter begins with the presentation of the annual variation of the physicochemical regulatory factors of phytobenthic associations, from the substrate type to abiotic factors (**subchapter 3.1.1.1**) (Table 1). The abiotic factors were analyzed based on the selection of data collected in 11 oceanographic expeditions performed during 2009-2019 within NIMRD Constanța. Thus, the data (N = 59) corresponding to warm season (May-September) were

selected from 6 stations (Constanta Nord, Casino, Eforie Sud, Costinesti, Mangalia and Vama Veche) at depths between 5 to 8 meters, adjacent to the phytobenthic sampling stations.

Table 1. Descriptive statistics of abiotic factors (2009 - 2019, warm seasons)

	Valid N	Mean	Median	Minimum	Maximum	Std. dev.
T [ºC]	58	21.80	23.05	12.20	27.80	4.07
S [‰]	58	14.24	14.32	7.61	18.91	2.60
O_2 [µM]	59	308.35	306.10	204.10	436.30	49.39
O_2 [%]	58	121.35	119.70	89.40	184.40	17.44
O_2 [mg/L]	59	9.86	9.79	6.53	13.96	1.58
$(PO_4)^{3-}$ [µM]	59	0.35	0.30	0.01	1.93	0.38
$(SiO_4)^{4+}$ [µM]	59	5.60	4.04	0.23	27.40	4.90
$(NO_2)^-$[µM]	59	2.58	0.30	0.05	42.26	7.23
$(NO_3)^-$ [µM]	59	7.90	3.28	0.01	69.23	12.27
$(NH_4)^+$ [µM]	59	6.95	5.90	0.37	33.10	6.18

As a particularity, the summer season 2010 was characterized by extremely high sea water temperatures (up to 28 - 29 ºC) which led to anoxic phenomena with serious repercussions on the marine ecosystem. Extremely high air temperatures led to coastal waters warming to rare values recorded along the Romanian Black Sea marine waters, reaching 26 – 28 ºC in upper layers. The exception was the extension of these extremely warm waters to offshore waters along Constanța profile, the registered temperature reaching values up to 25 - 27 ºC at over 35 km from the shore. As consequence, mass fish mortality phenomena, algal blooms and abundant developments of opportunistic macroalgae were recorded along the entire Romanian coast.

Based on the increase of water temperature and the influence of land pollution sources, as well as the intensification of tourism, during 2009 - 2019 summer seasons a decrease in phosphate concentrations comparing to the period of intense eutrophication was observed. However, these recorded values were still higher compared to the reference period 1959 - 1969. Inorganic nitrogen concentrations remained high especially near the land-based pollution sources. The increased amount of nutrients is the main responsible of the abundant development of macroalgae during the summer season. The response of macroalgae associations is correlated with environmental factors in the coastal zone. Thus, in an area with a high load of nutrients, intense anthropogenic activities and a high water turbidity, opportunistic species with a high development cycle will develop, unable to ensure a stable habitat for the associated fauna. If the degree of disturbance is extremely pronounced, it can reach to the total disappearance of phytobenthic associations, their place being taken by a bare and lifeless substrate.

The phytobenthic species list from the Romanian Black Sea coast identified in the last century (**subchapter 3.1.1.2**), was performed based on the analysis of over 100 articles from

1930 to 2019 and personal research, more than 90 years of phytobenthic research being analyzed. The species list is presented as an annex, next to each species being specified also the bibliographical reference(s) that attest its presence along the Romanian coast. Following the bibliographic synthesis, it can be said that only for 135 species there is concrete evidence of existence for the Romanian coast. Each species was validated by consulting the sites Algaebase (http://www.algaebase.org/) and WORMS (http://www.marinespecies.org/), in order to update the name of the species and to establish the degree of synonymy between the species.

Of the 36 species identified between 2009 and 2019 along the Romanian Black Sea coast, a small number of species were constantly reported at most monitored stations. This is the case of *Ulva* (*U. rigida*, *U. intestinalis*, *U. compressa*, *U. flexuosa*), *Cladophora* (*C. vagabunda*, *C. sericea*, *C. laetevirens*, *C. albida*) and *Ceramium* (*C. virgatum*, *C. siliquosum* var. *elegans*, *C. diaphanum*) species, the main components of the algal deposits formed along the shore during summer season. For all these opportunistic species, but also for the perennial key ecological important ones (*Cystoseira barbata*, *Phyllophora*, *Zostera noltei*), the morphological features were highlighted through 51 original photos that capture important qualitative macro and microscopic aspects (**subchapter 3.1.1.3**). The qualitative analysis is completed by the presentation of some rare species, identified as a result of constant monitoring. This is the case of the red algae *Lomentaria clavellosa*, *Dasya baillouviana* and *Spermothamnion strictum*, whose presence is particularly important for the phytobenthic biodiversity state, because over time they have become endangered, reaching the point of being considered extinct along the Romanian coast (case of *D. baillouviana* - Fig.2 and *S. strictum*). In fact, for *S. strictum*, the last report (and the only one by the way) dates from 1935, when it was reported by Maria Celan, and at the same time, *D. baillouviana* has not been identified since the 70s. The chapter has educational value and can be used as a guide in differentiating the main phytobenthic species from the Romanian coast.

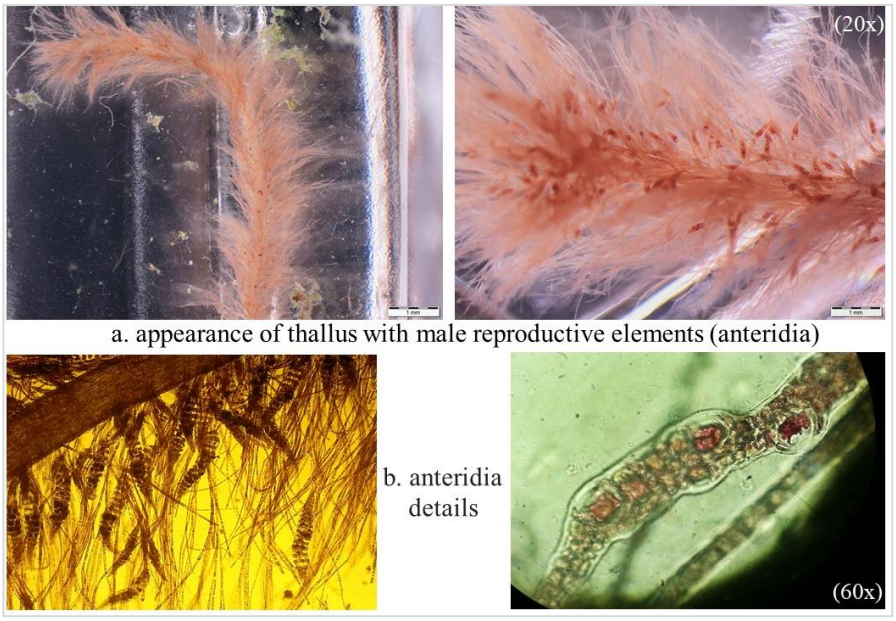


Fig.2. *D. baillouviana* – appearance of thallus with reproductive elements (original photo)

Subchapter 3.1.1.4. presents a detailed analysis of the current ecological status compared to that of the past decades, the conclusion being that the number of phytobenthic species has decreased progressively over time. On the Romanian coast, the largest number of species was reported between 1950 and 1960 (96 species). Also, a high number of species was observed during 1962 - 1972 (81 species) and 1970 - 1981 (81 species), after these periods there was a continuous qualitative decline (Table 2). The causing factors were natural, but especially anthropogenic, becoming more acute over the decades, turning into triggering factors of the qualitative decline of the phytobenthic component along the Romanian Black Sea coast.

Table 2. Number of species (by phylum) identified during 1930 - 2019

PHYLUM	1930-1950	1950-1960	1962-1972	1970-1981	1976-1995	1996-2005	2004-2007	2009-2019
CHLOROPHYTA	9	30	28	31	22	16	14	14
OCROPHYTA	8	22	17	12	9	4	5	4
RHODOPHYTA	27	44	36	36	24	10	9	14
TRACHEOPHYTA	-	-	-	2	-	-	1	4
TOTAL NO OF SPECIES	**44**	**96**	**81**	**81**	**55**	**30**	**29**	**36**

The '90s - early 2000s period is considered to be the period of maximum qualitative decline (Table 2), as a consequence of the intense eutrophication phenomenon with a negative influence on main perennial habitat forming species of the genera *Cystoseira*, *Phyllophora*, *Laurencia* and *Zostera*. Although incomparable to the qualitative richness of the past decades, nowadays a slight increase in species number can be noticed. This statement is valid only if we compare the current situation with that of the early 2000s. However, comparing the period 2009 - 2019 with that of the '50s -' 80s, the species number decline in such a relatively short period of time, becomes striking.

Phytobenthic communities were dominated until the 1980s by rhodophytes, followed by chlorophytes, generally represented by opportunistic species of the genera *Ulva* and *Cladophora*. The dominance of chlorophytes has been maintained for the last decade (Table 2), so most of the Romanian coastal zone is currently populated by macroalgal associations formed by opportunistic green algae with abundant development especially in summer season. The brown algae, along with the red algae, have suffered an acute decline on Romanian coast. Between 2009 and 2019, only 4 species of brown algae were reported, a significant reduced number compared to the maximum of 22 reported for the period 1950 - 1960. Regarding red algae, a number of 14 species have been identified, mostly represented by opportunistic species of the genus *Ceramium*, *Callithamnion corymbosum* or *Polysiphonia*, an incomparably smaller number taking into account the maximum of 44 species reported for the period 1950 - 1960, but slightly increase compared to the '90s.

The marine phanerogams research is deficient comparing to macroalgae, so the past decades references offer insufficient information on these phytobenthic components. In general, where information is available, refers only to the two *Zostera* species, respectively *Z. marina* and *Z. noltei*. During the study period (2009 - 2019), 4 phanerogams have been reported for the coastal area: *Z. noltei*, *Ruppia cirrhosa*, *Zannichelia palustris* and *Stuckenia pectinata*. But the main species, the one with the highest ecological value, namely *Z. marina*, has not been reported along Năvodari – Vama Veche coast in the last decade. The last reporting of this species dates back to the early 1980s. The consequence of the qualitative and quantitative decline of the red and brown macroalgae is the inclusion of a number of 10 species on the current Red List of endangered marine species, valid from April 9, 2020, based on Order no. 488/2020[1]. The two marine phanerogams mentioned above (*Z. noltei* and *Z. marina*) are also on the same list.

3.1.2. Quantitative distribution of submerged vegetation on the Romanian coast in the last decade

The analyzed period was the summer seasons 2009 - 2019, when is considered to be the period of maximum development for the phytobenthic vegetation along the Romanian coast. Currently, the phytobenthic communities are distributed at depths between 0 to 8 meters, the maximum wet biomass values being also recorded in this depth range. After 8 meters, is no longer possible to speak of continuous phytobenthic associations, but only of sparse macroalgal clusters. For the depth range of 0 to 8 meters, between 2009 and 2019, the maximum average wet biomass values varied between 3500 g/m^2 and 13400 g/m^2 (Fig.3).

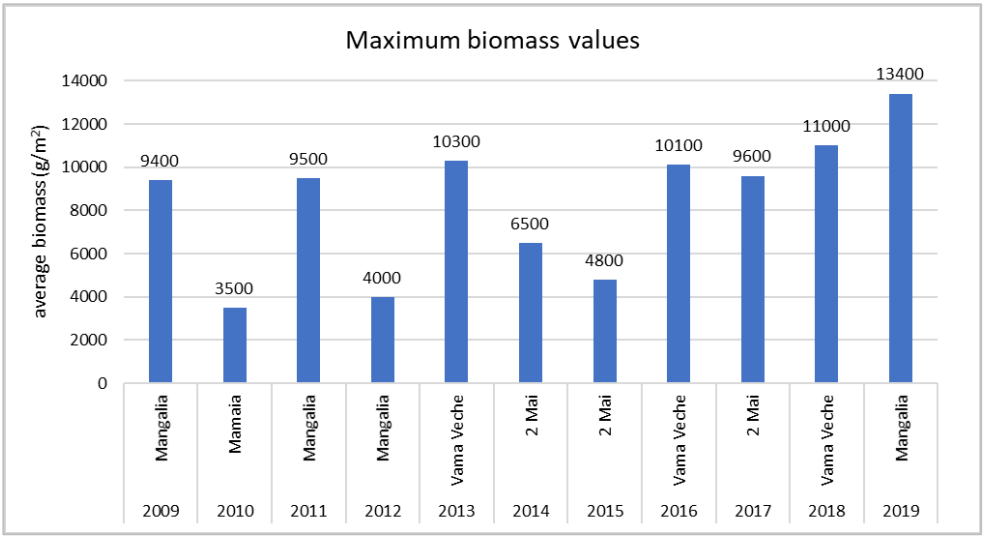


Fig.3. Wet biomass maximum average values recorded between 2009 - 2019

[1] Order no. 488/2020 on the approval of the List of endangered marine species on the Romanian Black Sea coast in order to protect and conserve them, published in the Official Monitor 300 of April 9, 2020.

Wet biomass multiannual variation (**subchapter 3.1.2.1.**) showed that the maximum biomass values were recorded towards the southern part, respectively along the coastal strip Mangalia - 2 Mai - Vama Veche. A clear gradient from north to southern part, in terms of increasing the biomass value from Năvodari to Vama Veche, was observed. In these southern areas, the dominant are the brown algae and marine phanerogams (Fig.4). However, we cannot talk about an exploitation of this biomass since is generated by an extremely reduced Red List species. In other areas, for the period 2009-2019, the opportunistic green algae of the genera *Ulva* and *Cladophora* were quantitatively dominant.

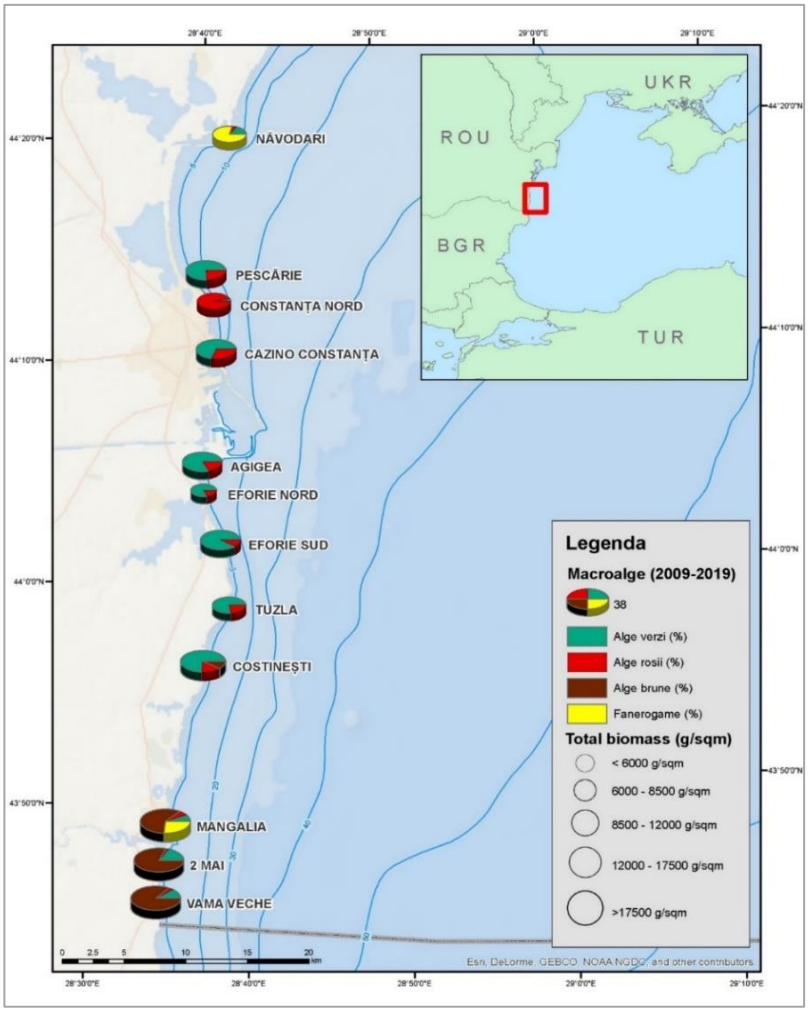


Fig.4. Biomass proportion of the phytobenthic components along the Romanian coast between 2009 and 2019

The similarity analysis between the monitored areas based on wet biomass values (**subchapter 3.1.2.2.**) showed high similarities, explainable due to the presence of a small number of phytobenthic species in the last decade, the permanent alternation of hard and sandy substrate along the entire coastal area, which imprints a uniformity character to the phytobenthic component. However, during the study period, a clear differentiation was observed for Mangalia

- 2 Mai - Vama Veche area from the rest of the monitored areas, in terms of biomass values and species diversity (Fig.5). The explanation consists in the existence of some compact platforms that allow the proper development of perennial species with much higher biomass values compared to seasonal species.

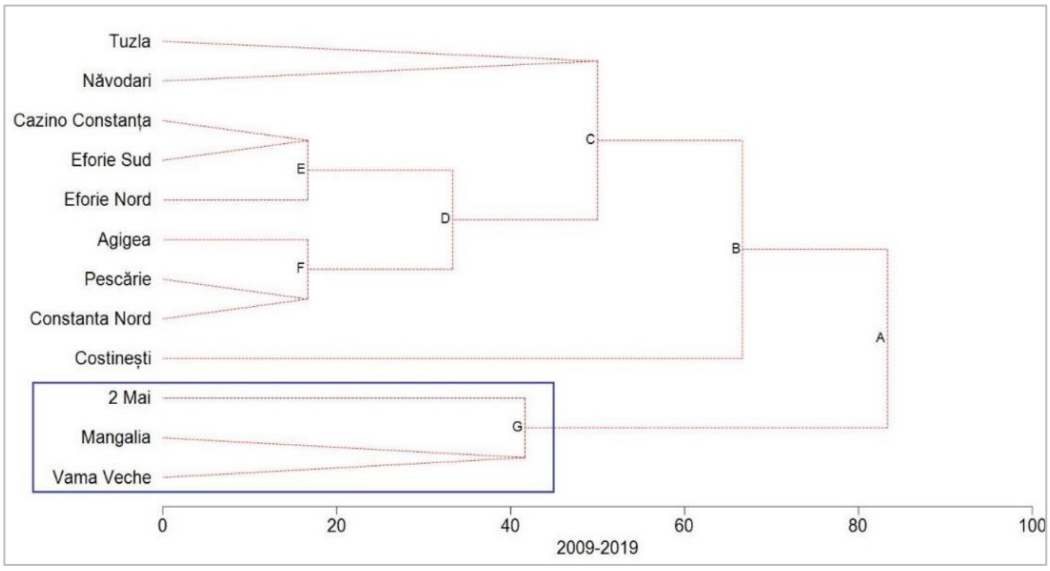


Fig.5. Linktree – sampling stations similarity based on wet biomass values for 2009 – 2019

Wet biomass multiannual variation for the dominant opportunistic species (**subchapter 3.1.2.3.**) showed the exclusive quantitative dominance of opportunistic species from *Ulva*, *Cladophora* and *Ceramium* genera. Among these, *U. rigida* and *C. sericea* developed more intense (Fig.6.).

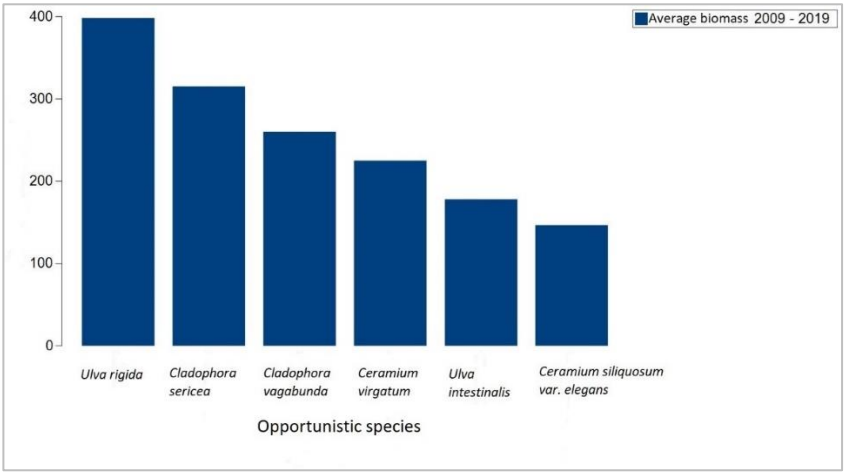


Fig.6. Average wet biomass variation for opportunistic species between 2009 and 2019

Regarding the quantitative evolution of opportunistic species, three scenarios have manifested in the last decade during the summer season. Thus, in the first scenario (reported in

2009) the dominant quantitatively genus was *Ulva*. The second scenario began in 2010 summer season, whose abnormal temperature, salinity values and nutrient concentrations proved to be extremely favorable for the intense development of *Cladophora* species. The third scenario has emerged since the summer of 2014, when the quantitative dominance returned to *Ulva* species. A new scenario seems to begin starting with 2019, when another increase in biomass values for *Cladophora* species was recorded. Nevertheless, if this opportunistic genus will experience the same massive development as in previous years, it remains to be confirmed or refuted by future research.

The comparative analysis of the study areas according to the annual evolution of wet biomass for opportunistic species (**subchapter 3.1.2.4.**) showed that during the warm season, green algae have been quantitatively dominant in the last decade, a characteristic of the Romanian coast. Mass development for species with a short vegetation period have been observed since the 70s (Țigănuș, 1979). Comparing all dominant qualitatively and quantitatively opportunistic species, we first observe their constant presence along all sampling stations from Năvodari to Vama Veche. This fact imprints and supports the uniformity of phytobenthic character that is currently noticeable along the Romanian coast.

The MDS configuration shows a 70% similarity between all 12 constantly monitored stations during the 11 years. Constanța Nord is obviously detached (Fig.7). The explanation lies in the specificity of the area. At Constanța Nord there is a reduced rocky platform, interrupted by sandy areas, on which *Coccotylus brodiaei* (a perennial Red List species with special ecological value, related to *Phyllophora* species), is currently developing. In this area, opportunistic species have not developed high biomass values compared to other coastal areas.

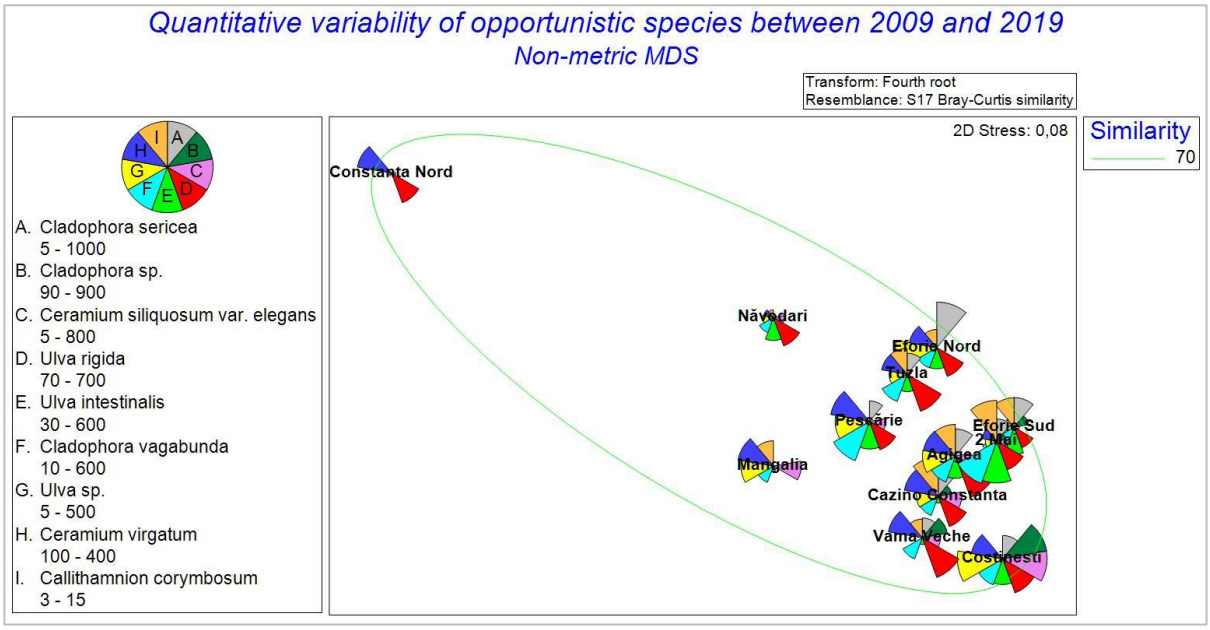


Fig.7. Two-dimensional MDS configuration of dominant opportunistic species for the period 2009 - 2019 (4th root biomass transformed values)

The thesis also analyzes from a quantitative point of view the perennial key ecological role species, respectively the habitat forming species, *Cystoseira barbata*, *Coccotylus brodiaei* (**subchapter 3.1.2.5**.) and *Zostera noltei* (**subchapter 3.1.2.6**.). All these are endangered species and require a constant monitoring to detect any changes in the populational structure.

Currently, *C. barbata* distribution is scatter, with fragmented populations only in the southern part, from Saturn to Vama Veche. This species quantitative analysis was performed in accordance to historical data. The reference period was considered to be 1969, before a series of extreme phenomena, which led to the destruction of *Cystoseira* populations: sea ice from 1971 - 1972 winter, strong storms, low water temperatures that disrupted the photosynthetic activity and reproduction process. The average wet biomass value recorded in 1969 was 10600 g/m^2, while the current study period (2009 - 2019), average biomass was much lower, at half of these value, respectively 5750 g/m^2 (Fig.8).

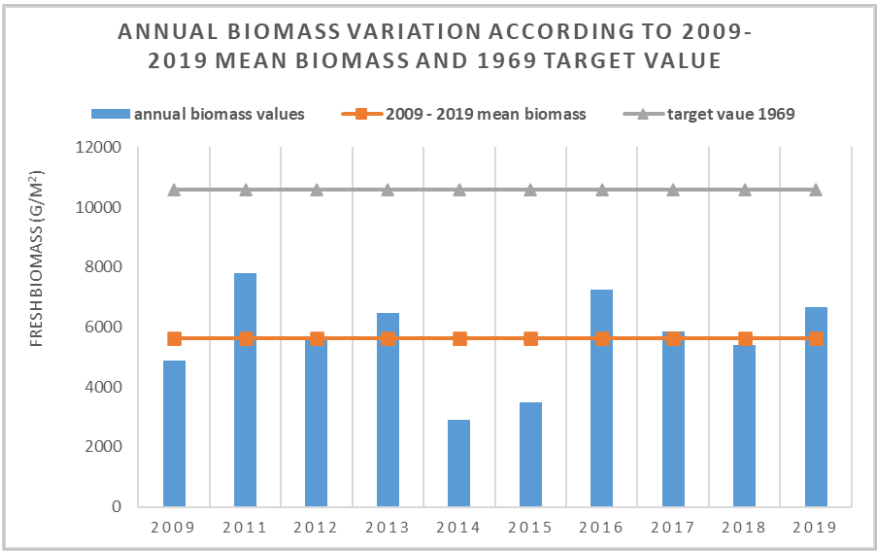


Fig.8. *C. barbata* – annual variation of the average wet biomass comparing to 1969 target value

Comparing the past decades situation with the present one, it is noticed that the current biomass values are much lower compared to those of the '60s, but much higher compared to the period of maximum decline 1971 – 1972, hence the regeneration hypothesis of this species along the Romanian coast (Fig.9).

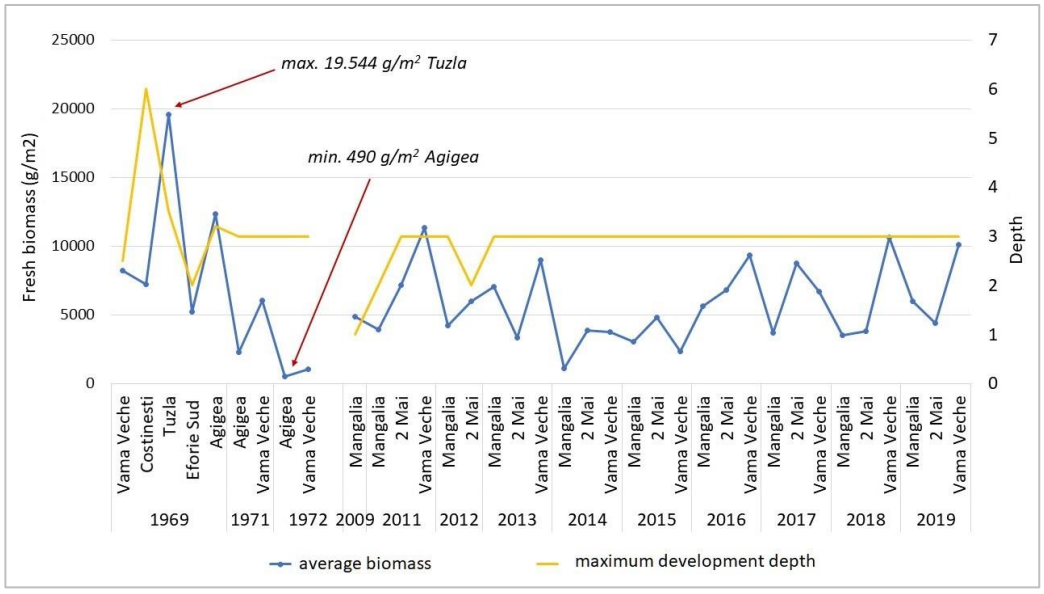


Fig.9. *C. barbata* – variation of development depth and average wet biomass during the study period 2009 - 2019 compared to historical data

Bray - Curtis analysis based on average biomass values shows two scenarios regarding the quantitative evolution of *C. barbata* along the Romanian coast over the decades:

- ✓ a high similarity (of 70%) regarding the last decade situation, respectively a low variability of biomass values between 2009 and 2019.
- ✓ at the opposite side, there are the situations from 1971 - 1972 (considered the period of drastic decline for *Cystoseira* populations, with extremely low biomass values), respectively the situation of 1969 (considered the reference period), stages with no similarity within the current environment conditions (both quantitative and qualitative) (Fig.10).

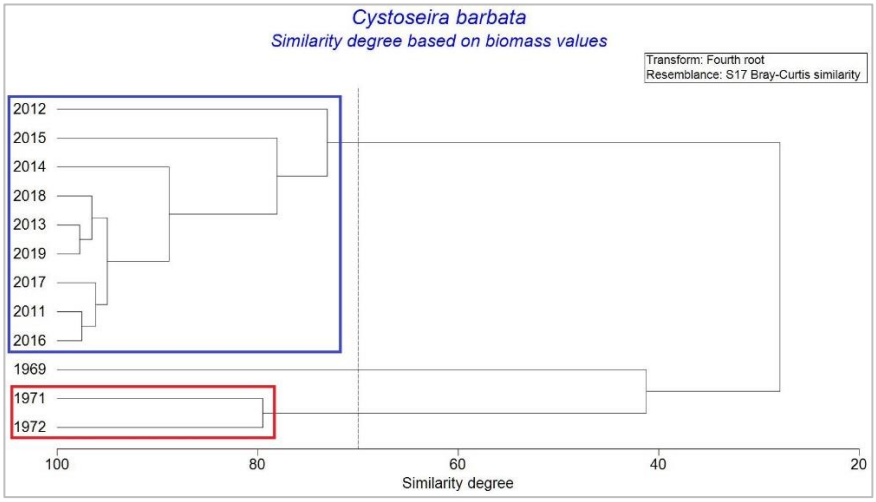


Fig.10. *C. barbata* - 2009 - 2019 Bray-Curtis similarity compared to 1969 - 1972 (4th root biomass transformed values)

The last decade conclusion regarding this species, is that the lowest biomass values were recorded at Mangalia, and the highest at Vama Veche. Based on wet biomass values, ANOVA Single Factor was applied to test the differences between the three data sets (Mangalia, 2 Mai and Vama Veche) for the period 2009 - 2019. The statistical obtained results show that although the biomass values are different between the three sampling stations during the last decade, the biomass variability is reduced within each station. The fact that the species biomass remained relatively constant for the last 10 years, is a particularly important aspect, considering the sensitivity of this species to anthropogenic disturbing factors.

Of the three species of *Phyllophora* reported for the Romanian coast (*P. pseudoceranoides, Coccotylus brodiaei* and *P. crispa*), quantitative data is available only for *C. brodiaei*. The species is rare, included in the Red List, with a scatter distribution (for the moment only at Constanța Nord, between 6 to 8 meters depth), with a variable wet biomass and a slightly increasing trend in 2019.

Regarding the marine phanerogams, from the four known species (*Zostera noltei, Stuckenia pectinata, Ruppia cirrhosa, Zannichellia pallustris*), the quantitative evaluation was performed for only *Z. noltei* and *S. pectinata*. The other two phanerogams do not form stable communities in shallow coastal waters, only scattered specimens being reported. *Zostera* populations were reported at Năvodari and Mangalia, at depths between 1 to 3 meters. At both stations the tendency of biomass decreasing was observed during the monitoring period. The multiannual biomass evolution shows a decrease gradient, and the average value was not reached in the past seven years (Fig.11).

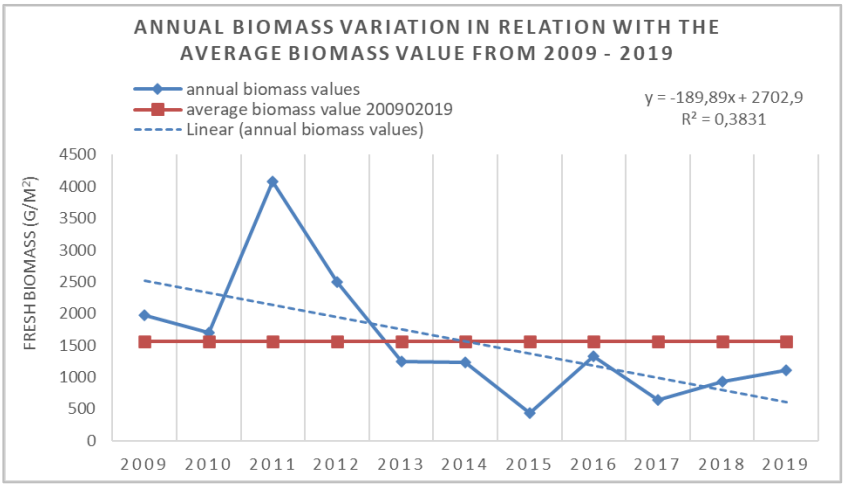


Fig.11. *Zostera noltei* – multiannual biomass wet variation comparing to the average value for 2009 - 2019 period

As in the case of *C. barbata*, also for *Z. noltei*, ANOVA Single Factor was applied to test the differences between the two data sets (Năvodari and Mangalia) recorded during 2009 - 2019. It showed that the biomass values for *Z. noltei* did not differ significantly between the two sampling stations, the tendency of decreasing biomass values being common to both stations.

3.2. THE INFLUENCE OF ANTHROPOGENIC CONSTRUCTIONS ON PHYTOBENTHIC COMMUNITIES

3.2.1. Consequences of recent coastal protection constructions on submerged vegetation

This chapter presents information on the qualitative and quantitative structure of macroalgal communities developed on the 11 newly built coastal protection dikes (9 dikes in Mamaia - Constanța and 2 dikes in Eforie Nord area), along with information regarding the physicochemical parameters variability (Fig.12). In July, near Constanța dikes, high temperature values were reported (between 23.2 and 24.9˚C), and the oxygenation conditions showed oversaturation. Increased salinity values (17,19 - 17, 69 PSU) were also reported due to evaporation, lack of precipitation and low Danube flows recorded during that period (Fig.12). For the beginning of August, the high water temperature values were maintained, with maximums values up to 27.8˚C. In general, oxygen deficiency was observed near all dikes.

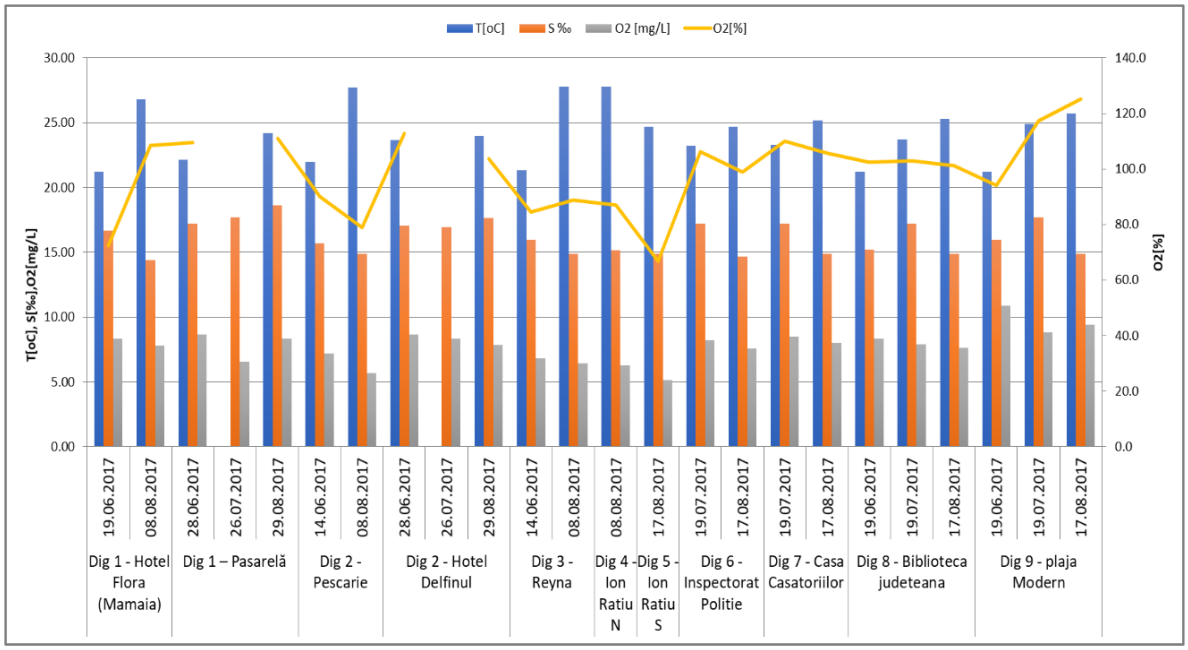


Fig.12. Spatial variability of physicochemical parameters (summer 2017)

The qualitative analysis of the samples led to the identification of 12 macroalgal species along the newly built coastal protection dikes. The clear dominance of chlorophytes was observed both in Constanța and in Eforie Nord, the first species ready to populate the new available substrate being *Ulva* sp. and *Cladophora* sp. These species have a pronounced opportunistic character and are resistant to desiccation (Fig.13).

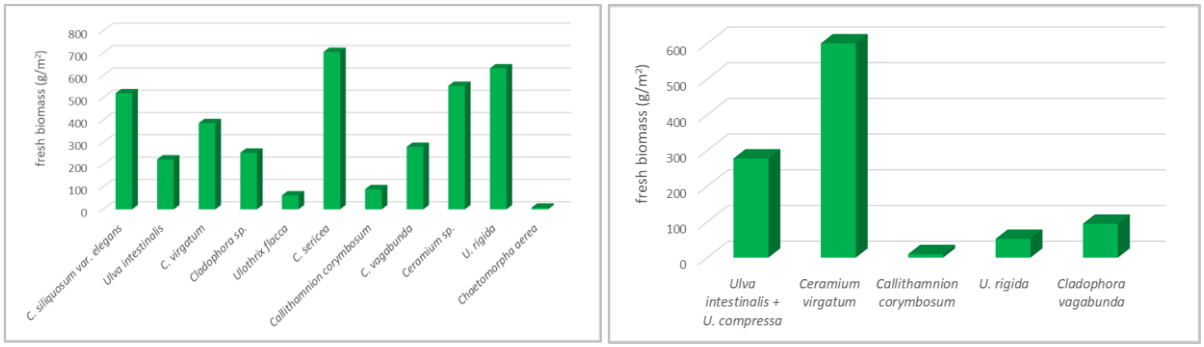


Fig.13. Wet biomass variation for the dominant species along new dikes from Mamaia - Constanța (left) and Eforie Nord (right)

The qualitative analysis highlighted near Constanța the presence of *D. baillouviana*, a species considered extinct from the Romanian Black Sea coast, included in the Red List of endangered marine species.

3.3. PHYTOBENTHIC COMMUNITIES AS INDICATORS OF ECOLOGICAL STATUS FOR THE COASTAL MARINE ENVIRONMENT IN ACCORDANCE WITH THE PRINCIPLES OF MARINE STRATEGY FRAMEWORK DIRECTIVE

The assessment of the ecological status of coastal water bodies and coastal habitats was performed using the Ecological Evaluation (EI) index, based on the principle of reporting the obtained values at a "threshold value", according to Marine Strategy Framework Directive principles. The threshold value represents, within the meaning of the Decision (EU) 2017/848 of 17 May 2017, a value or series of values that allows the assessment of the quality level ensured for a certain criterion, thus contributing to the assessment of achieving good ecological status (DECISION (EU) 2017/848 of 17 May 2017). Achieving this goal (namely achieving or maintaining good ecological status in marine environment) obliges Member States, including Romania, to make every effort to adopt the best protection and conservation measures in their own marine region, namely the Black Sea for Romania (https://ec.europa.eu/environment/marine/good-environmental-status/index_en.htm).

Ecological Evaluation is a multiparametric index based on the proportion between sensitive and tolerant to eutrophication conditions species, and includes information on wet biomass and specific diversity (Dencheva and Doncheva, 2014; Berov *et al.*, 2018).

3.3.1. The results of the Ecological Evaluation (EI) index application used for the assessment of the ecological status of coastal water bodies

The assessment of the ecological status was performed for the two natural coastal water bodies (established from Periboina to Cap Singol and from Eforie Nord to Vama Veche) and

for the modified water body (between Cap Singol and Eforie Nord), by analyzing the ecological status of the phytobenthic communities from various stations belonging to these water bodies. Following the analysis, during 2009 - 2019, a slight trend of improving of the ecological status was observed from northern to southern part, respectively from the Periboina - Cap Singol water body to Eforie Nord - Vama Veche water body (Fig.14).

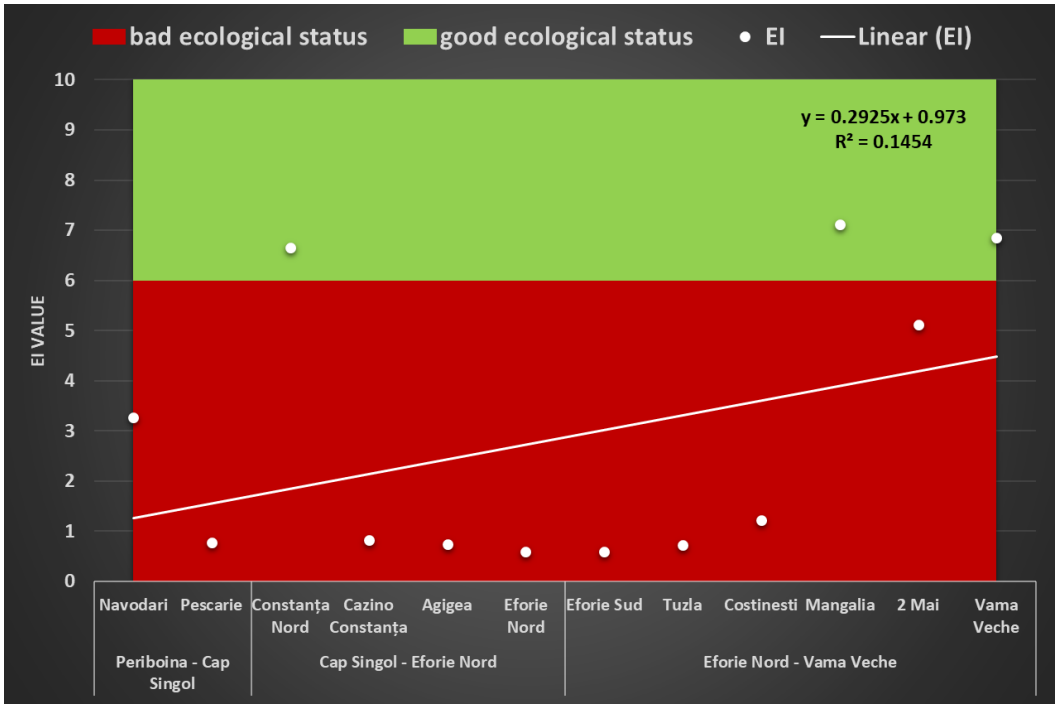
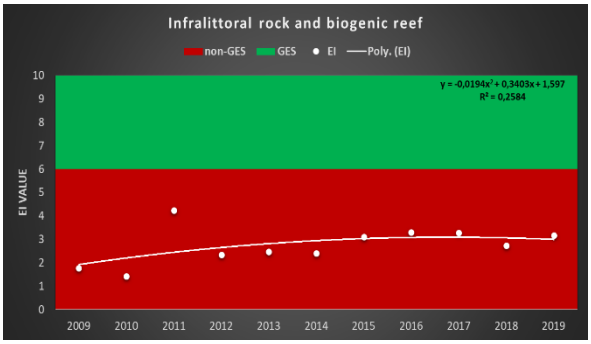


Fig.14. Coastal water bodies ecological status between 2009 and 2019

3.3.2. The results of the Ecological Evaluation (EI) index application used for the assessment of the ecological status of coastal habitats

For the infralittoral part of the Romanian Black Sea coast, two broad habitat types (Infralittoral sands and Infralittoral rock and biogenic reef) and four special habitats, sub-types of the broad habitat types mentioned above (Habitat with *Zostera noltei*, Infralittoral rock with photophilic algae, Habitat with *Coccotylus brodiaei* and Habitat with *Cystoseira barbata*), according to the EUNIS classification (COMMISSION DECISION (EU) 2017/848 of 17 May 2017).

The annual ecological assessment for the broad habitat Infralittoral rock and biogenic reef shows a bad ecological status. The other broad habitat, Infralittoral sands, was in good ecological condition in proportion of 61% along the entire coastal area during the evaluation period (Fig. 15).



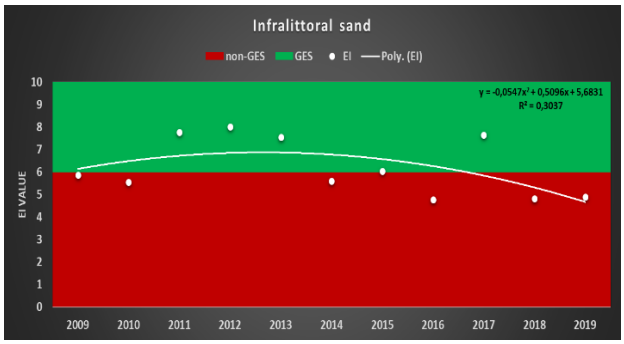
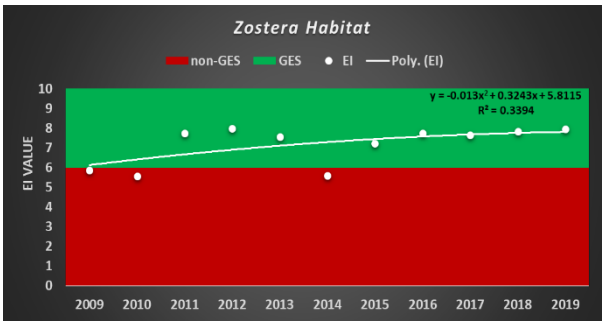
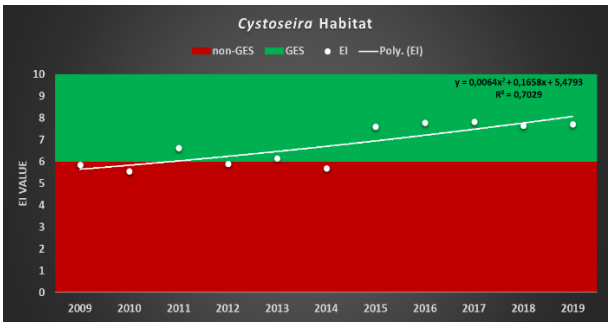


Fig.15. The evolution trend of the ecological status of the major Infralittoral rock and biogenic reefs and Infralittoral sands habitats in the 2009 - 2019 period

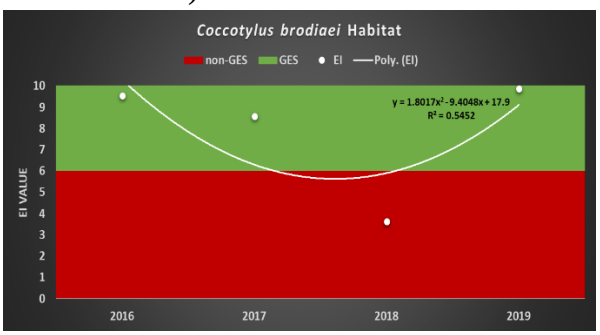
The special habitat with *Zostera noltei* (important ecological habitat of European interest) was in good ecological status in proportion of 80% during the study period, observing the maintenance of good ecological condition in the last five years (Fig.16 a). The evaluation showed a clear trend of improvement in the ecological status for *Cystoseira barbata* habitat in the last five years (Fig.16 b). This habitat was in good ecological condition in proportion of 75% during the evaluation period. The habitat with *Coccotylus* was in proportion of 75% in a good ecological status during 2016 - 2019, but a constant monitoring is necessary to see the evolution of this habitat (Fig. 16 c). Considering the scatter distribution of all these special habitats along the Romanian coast and the endangered species included in the Red List status of all mentioned above species, it can be stated that the ecological status of these habitats is satisfactory.



a) *Zostera* habitat



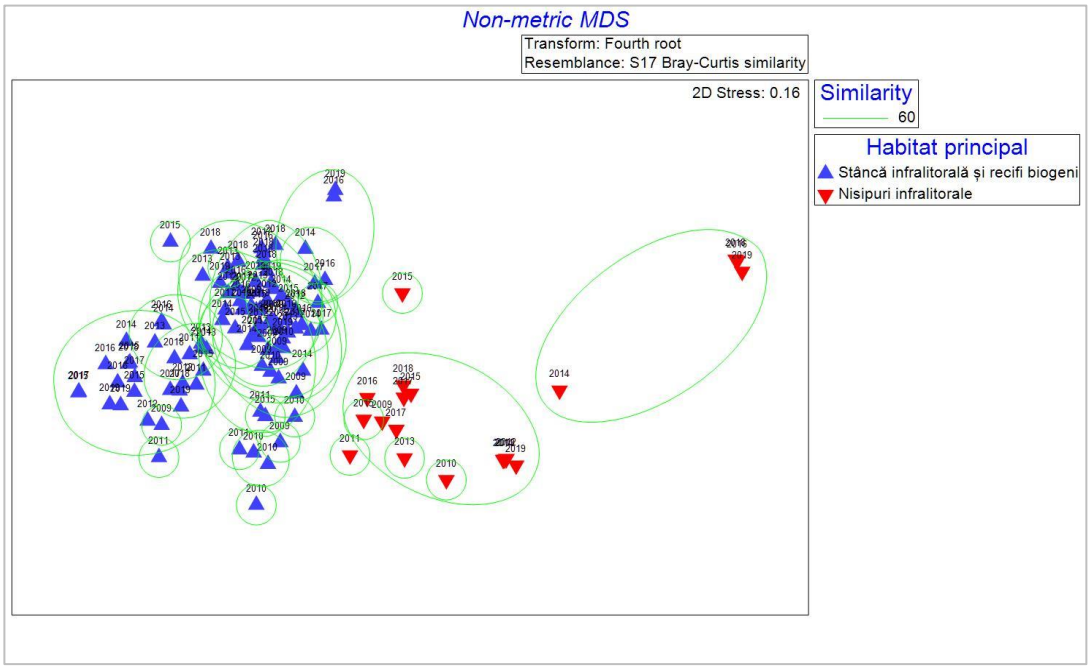
b) *Cystoseira* habitat



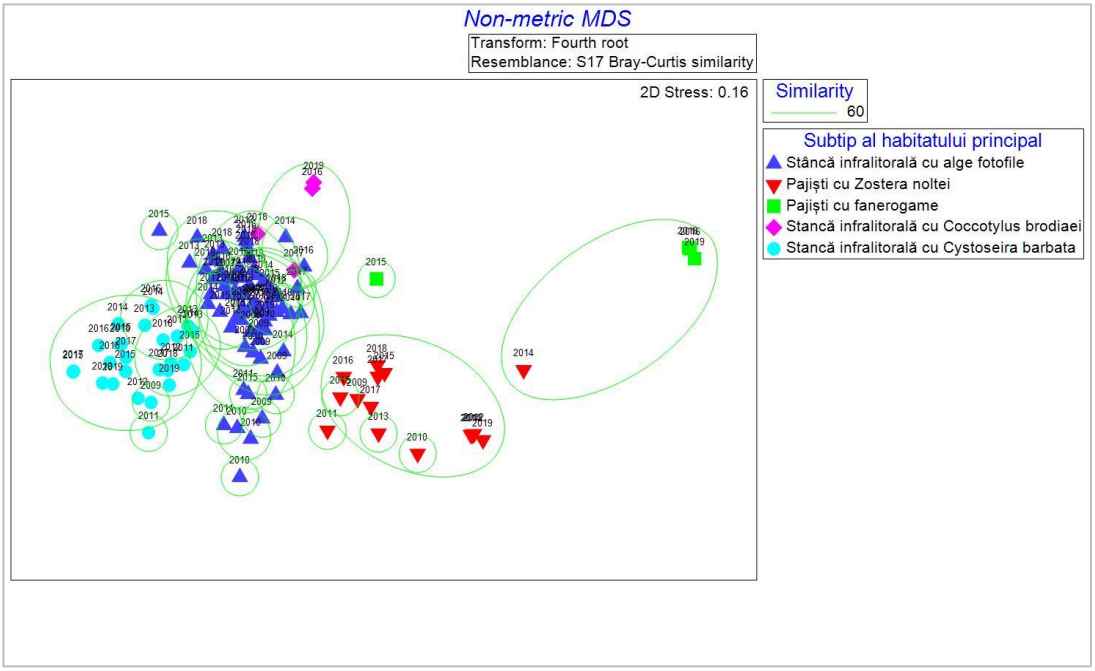
c) *Coccotylus* habitat

Fig.16. Ecological status for the special habitats type between 2009 - 2019

Analyzing the annual situation of the phytobenthic communities within the broad habitat, but also of their subtypes, it is observed that there were no major differences regarding the ecological status of the phytobenthic associations within these habitats. A constant situation regarding the qualitative and quantitative evolution of phytobenthic species was maintained during 11 years of constant monitoring (Fig.17).



a) Broad habitat type



b) Subtypes of broad habitats

Fig.17. Phytobenthic communities' similarity within coastal habitats (2009 – 2019)

3.4. RESULTS OF MACROALGAL CULTURES CARRIED OUT UNDER LABORATORY CONTROLLED CONDITIONS

Globally, seaweed cultivation is an industry that has expanded rapidly in recent years, mainly due to market demand that has exceeded the supply of available natural resources. However, macroalgal laboratory culture is a new domain along the Romanian coast. This method can provide raw algal material with exploitable potential in the most diverse fields, without an exploitation and inevitable endangerment of natural resources. At the same time, from a strictly biological point of view, macroalgae cultures provide interesting information on the intermediate stages of development and reproduction of algae, aspects that cannot be captured in natural environment of life. This last chapter of the thesis presents the interesting results obtained after performing macroalgal cultures under laboratory-controlled conditions for two species – the stenothermic red alga, *Porphyra leucosticta* and the perennial brown alga, *Cystoseira barbata*. All the steps, from the collection of biological material from natural environment to the obtaining of new individuals exclusively under laboratory-controlled conditions, were illustrated with 85 original photos. The conclusions of the experiments were that both species are suitable for laboratory-controlled cultures in order to obtain algal material for various purposes (valorification or ecological reconstruction).

3.4.1. Culture results for the red alga *Porphyra leucosticta*

After collecting the donor biological material from the natural environment, in the laboratory were carried out various steps of analysis the algal material, processing and assembling the experiment (Redmond *et al*., 2014). Spores germination and formation of new blades (with an initial division on a single plane), were observed after 5 days. After 3 - 4 weeks from the beginning of the experiment, the development of numerous juvenile specimens was observed, which became macroscopically visible (approximately 0.2 - 0.4 cm in length) after about a month (Fig.18).

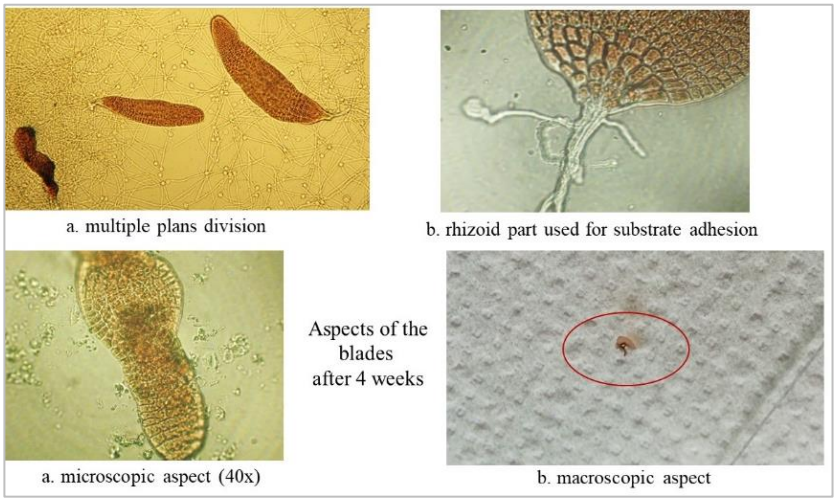


Fig. 18. *P. leucosticta* – Aspects of culture thallus (after 4 weeks of experiment)

After 5 weeks, an interesting phenomenon was observed, namely a new release of spores from the newly formed blades and the appearance of various specimens in an early form of development. In other words, the blades obtained in culture became reproductive. This is considered the 2nd generation of specimens, being a way of continuously obtaining algal material. The new specimens became macroscopically visible after another 3 weeks. After 6-7 weeks, the size of the newly formed algae under laboratory culture varied between 2 mm and 1.5 cm. (Fig.19).

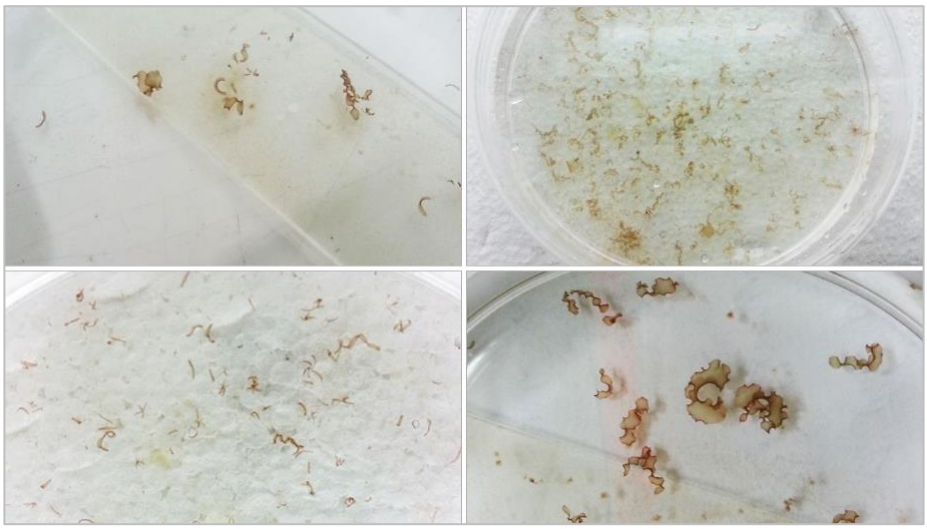


Fig.19. *P. leucosticta* – macroscopic aspects of the obtained blades after 2-3 months in laboratory-controlled culture

After 3 months, the culture with specimens of approximately 1 cm in length was transferred to 250 ml Erlenmeyer flasks, aeration was introduced and the photoperiod was set at 12:12 (12 h of light and 12 h of dark - i.e. neutral day). The temperature was set at maximum 15˚C, all these being necessary conditions for accelerating the thallus growth (Redmond *et al.*, 2014) (Fig.20).

Fig.20. Aspects of laboratory-controlled culture of *P. leucosticta*

Towards the end of the experiment, the maximum size of the thallus reached up to 8 cm (Fig. 21), the wet biomass of culture algae was 44.23 g, and the dry biomass (after lyophilization) was 4.94 g.

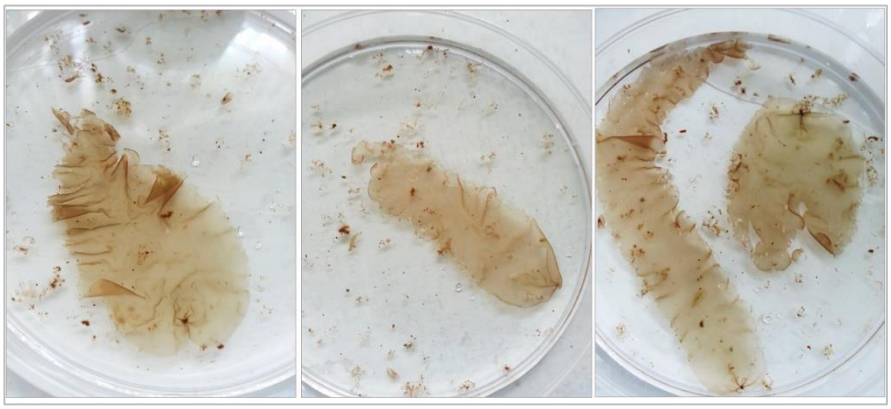


Fig.21. Aspects of *P. leucosticta* blades grown in laboratory after 3 months of experiment

3.4.2. *Ex-situ* culture results for the brown algae *Cystoseira barbata*, included in the Red List of endangered marine species

Given its condition as an endangered species, the restoration of *C. barbata* populations along the Romanian coast would involve relocation operations, respectively the relocation of mature individuals from donor areas with natural populations to other areas. However, given the critical conservation status of *Cystoseira* populations and the low availability of biological material, less invasive techniques are recommended (Verdura *et al.*, 2018). Obtaining *C. barbata* new thalli under controlled culture is considered to be such a non-invasive technique and a possible solution to control this species decline along Romanian Black Sea coast. In order to follow the evolution of the newly formed thalli for as long as possible and to create an overview of the whole process, the culture spread for over 13 months. The experiment involved two scenarios:

- ✓ An initial scenario carried out during the warm season of 2019 (from June till September), when the experiment took place outdoors, under natural light conditions, to simulate as close as possible the natural environmental conditions. This aspect was absolutely necessary in the early extremely sensitive life stages of development.
- ✓ A secondary scenario started after 16 weeks from the beginning of the experiment and coincided with the decrease of the atmospheric temperature with the installation of the cold season. Thus, the culture was moved to an enclosed space (laboratory), where it was monitored until the end of the experiment.

After collecting the fertile apical branchlets from the natural environment (Mangalia area), the experiment was assembled (Verdura *et al.*, 2018). One week after the start of the experiment, embryos of the future *C. barbata* thalli in early stage of development were already

observed. Four incipient stages of development are defined for *C. barbata* (Falace *et al.*, 2018). Initially, the first two stages of development of the species were reported (the round-shaped and the elongated form) (Fig. 22).

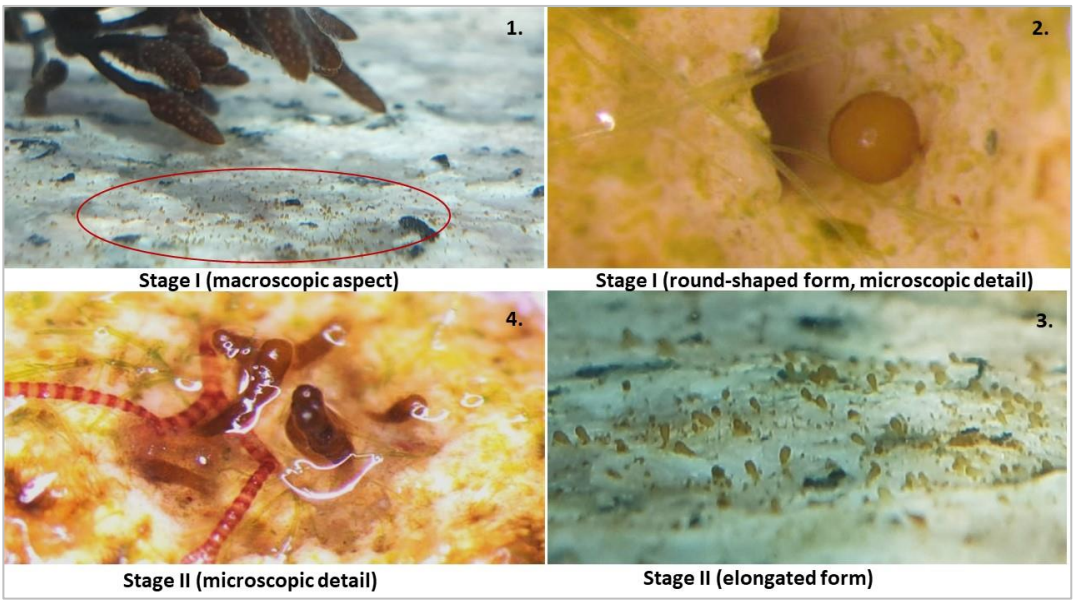


Fig.22. *C. barbata* - early stages of development in laboratory culture (stages I and II)

After 40 days, the appearance of stage III development is noticed, the elongated with branching form, in other words, the one with the first ramifications. The development degree of the new thalli is various, the size of the specimens varies between 0.5 cm and 1.5 cm, but it can certainly be said that all germlings are macroscopically visible after one month. After 2 months, the presence of stage IV (elongated with multiple branches) was noticed (Fig.23).

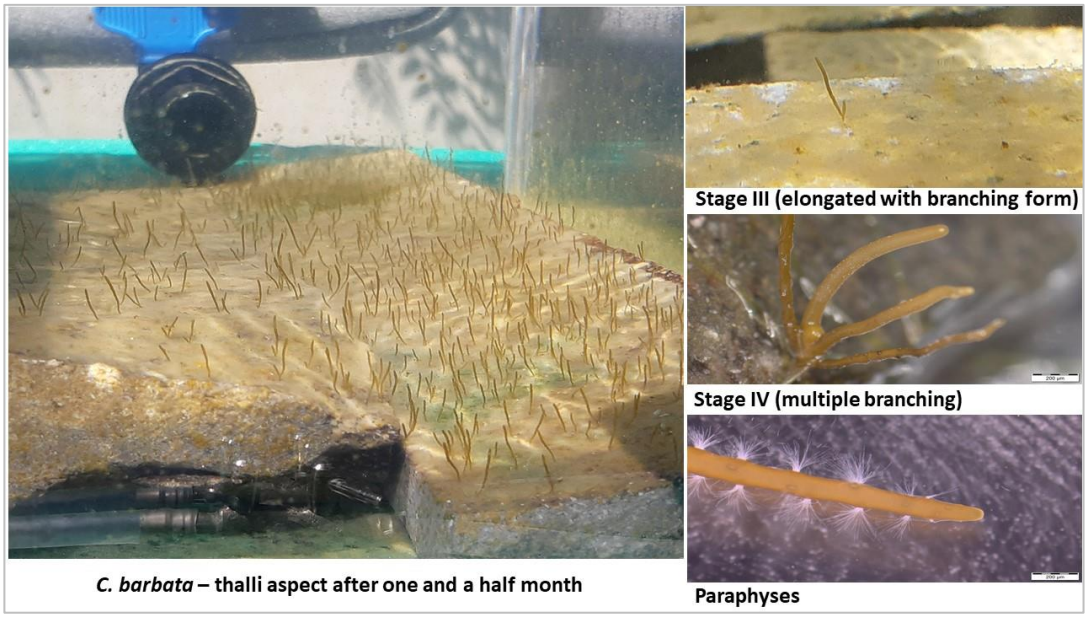


Fig.23. *C. barbata* – various stages of development in laboratory culture (stages III and IV)

After 2 and a half months, some of the fully developed recruits have 3 cm length and multiple branches. The basal fixation part is well defined. After 16 weeks, the recruits were placed under controlled conditions of light and temperature, where they were constantly monitored. Thus, after 1 year of experiment, 1220 new recruits of *C. barbata* were obtained exclusively in controlled culture, with dimensions that varied between 5 to 10 cm in height (Fig.24).

Fig.24. Variable dimensions of *C. barbata* recruits obtained in controlled laboratory culture

4. CONCLUSIONS

A number of 789 samples collected from the coastal area is basis for the elaboration of this thesis, from all of the 14 sampling stations located along the coastal strip Năvodari - Vama Veche, from depths between 0 to 8 meters. The following conclusions were formulated, following the research carried out between 2009 and 2019 and the interpretation of the obtained results:

1. The phytobenthic species list from the last decade was performed based on the analysis of over 100 bibliographic titles from 1930 till 2019 and personal research. The evidence of 135 phytobenthic species was reported for the Romanian coast, for the last 90 years.
2. Currently, the phytobenthic associations are well represented qualitatively and quantitatively only between 0 to 8 meters depth, after this range only sparse individuals being reported and not well-structured algal communities.
3. 36 species were identified during the study period, which represents only 37.5% of the total algal inventory from 1950 to 1960, considered the period of maximum development for the macroalgal species along the Romanian coast. The number of species reported during the research period, although reduced, is slightly higher than that reported number for the ‘90s - early 2000s (29 species), considered the period of

maximum decline. This aspect is mentioned to emphasize the fact that currently the submerged vegetation is in a slightly regeneration process along the Romanian coast.

4. From a qualitative point of view (in reference to species number), the phytobenthic component has progressively become impoverished over the decades, the triggering factors being both natural (extreme weather conditions) but especially anthropogenic (various anthropogenic activities in the coastal area).
5. Phytobenthic communities were dominated by the red algae until the 1980s, after which green algae dominated, generally represented by opportunistic species of the genera *Ulva* and *Cladophora*. The species of brown and red algae have been the most affected over decades along the Romanian coast, hence the small number of species identified nowadays.
6. Between 2009 and 2019, 14 green algae, 4 brown algae, 14 red algae and 4 marine phanerogams were identified.
7. Regarding the chlorophytes, the opportunistic species of *Cladophora, Bryopsis* and *Ulva* dominated qualitatively and quantitatively during the study period. Regarding the rhodophytes, the opportunistic species of the genus *Ceramium* were dominant, *Callithamnion corymbosum* and *Polysiphonia*.
8. For 2009 - 2019, the following marine phanerogams have been reported: *Zostera noltei, Ruppia cirrhosa, Zannichelia palustris* and *Stuckenia pectinata*. However, the species with the greatest ecological value, namely *Z. marina*, has not been reported along Năvodari – Vama Veche in the last decade. The last report for this species dates back to the early 1980s. Only the related species, *Z. noltei*, was identified at Mangalia and Năvodari, at depths between 1 - 3 meters.
9. No potentially invasive species were reported during the study period. The only potential invasive species, namely the brown alga *Desmarestia viridis*, was only a random presence reported between 2005 - 2006, but did not acclimatize and did not have a subsequent evolution along Romanian coast.
10. In the last decade, the restoration of *Cystoseira barbata* fields has been recorded at Saturn, Mangalia, 2 Mai and Vama Veche). The distribution area for this species is between 1 to 4 meters depth, with an optimum development depth range between 1.5 to 3 meters.
11. Nowadays, under current environmental conditions, in terms of ecological value, *Phyllophora* species are considered to be the most important representative of rhodophytes along the Romanian coast. Thus, *Coccotylus brodiaei* (syn. *Phyllophora brodiaei*) was reported at Constanța North area, with a distribution between 6 to 8 meters depth. *P. crispa* was identified at Sf. Gheorghe, at a depth of 30 to 35 meters.
12. The red alga *Spermothamnion strictum*, whose only report dates from 1935 (when it was first identified by Maria Celan) was reported at Sf. Gheorghe, in 2017, in association with *P. crispa*.

13. Following the quantitative analysis, the maximum biomass values were generally recorded towards the southern extreme part of the Romanian shore, respectively along the coastal strip Mangalia - 2 Mai - Vama Veche. A clear gradient could be observed during the study period, from the northern to southern part, in other words an increase in wet biomass values from Năvodari to Vama Veche.

14. During the summer season, when it is considered to be the period of maximum development for the submerged vegetation for the Romanian coast, the opportunistic species *Ulva*, *Cladophora* and *Ceramium* developed abundantly, also main components of the algal deposits. This mass development is a consequence of seasonal conditions - high water temperature and increased amount of nutrients. The fact that they reach the shore is a result of storms activity, currents and waves that pull the specimens from the substrate. All these correlated aspects have therefore as a consequence the formation of those algal deposits generating discomfort for tourists.

15. Between 2009 - 2019, for *C. barbata* the lowest biomass values were reported at Mangalia, and the highest at Vama Veche. The statistical obtained results (applying ANOVA Single Factor test) show that for the past decade, although the biomass values are different between Mangalia, 2 Mai and Vama Veche, the variability of these biomass values are reduced within each sampling station. The fact that the biomass remained relatively constant within each station, is a particularly important fact considering the high sensitivity degree of *C. barbata* to anthropogenic disturbing factors.

16. For *Z. noltei*, the statistical analysis (applying ANOVA Single Factor test) showed that the biomass values did not differ significantly between Mangalia and Năvodari during 2009 - 2019 study period. However, there was a tendency in decreasing the biomass values in both stations during the study period.

17. For *C. brodiaei*, biomass values were variable, with an increasing tendency in 2019.

18. Regarding the phytobenthic communities from newly built dikes along Mamaia - Constanța and Eforie North, a high specific uniformity was noticed, with a more abundant seasonal development of *Ulva* species (among green algae) and *Ceramium* (among red algae). 12 macroalgal species have been reported, generally opportunistic green algae. The exception was the identification in 2019, around the newly built dike near Constanța of the rhodophyte *Dasya baillouviana* (syn. *Dasya elegans*), a species considered extinct along the Romanian coast, currently included in the Red List of endangered marine species.

19. Submerged vegetation is a useful tool for establishing the ecological status of Romanian coastal waters in accordance with the requirements of the Marine Strategy Framework Directive. Accordingly, during 2009 – 2019, there was a slight tendency in improving the ecological status from north to extreme south, respectively from Periboina - Cap Singol coastal water body to Eforie North - Vama Veche. For the ecological assessment of coastal habitats, the following were noted:

- ✓ The broad habitat Infralittoral rock and biogenic reef was in a bad ecological status during the study period.
- ✓ The broad habitat infralittoral sands was in good ecological condition in proportion of 73% of during the evaluation.
- ✓ The special habitat with *Zostera*, subtype of the major type Infralittoral sands, was in good ecological status in a proportion of 90%.
- ✓ *Cystoseira* habitat, subtype of the broad habitat Infralittoral rock and biogenic reef, was in good ecological status in a proportion of 82% during the evaluation period.
- ✓ *Coccotylus* habitat, another subtype of Infralittoral rock and biogenic reef, was found in good ecological status.

20. The experiments carried out during the study period regarding the culture under laboratory-controlled cultures of two important macroalgal species, showed very interesting results:

- ✓ *Porphyra leucosticta* is a stenothermic red alga, with a high reproductive capacity and a high growth rate, which has proven to be suitable for laboratory culture. After 5 months of experimental culture, the obtained algal biomass was collected and weighed: wet biomass was 44.23 g; dry biomass was 4.94 g. The newly obtained blades had maximum dimensions of up to 8 cm.
- ✓ *C. barbata*, the species included in the Red List of endangered marine species, is also suitable to be cultivated under laboratory-controlled conditions for the future needs of ecological restoration along the Romanian Black Sea coast. After one year of experiment, more than 1200 new recruits of *C. barbata*, with a maximum height of 10 cm, were obtained exclusively in laboratory culture.

21. The major changes suffered by the phytobenthic community over decades along the Romanian Black Sea coast (some of them still continuing) are considered to be: the drastic reduction of species number, the disappearance of sensitive perennial species due to the their inability to adapt to degraded environmental conditions, the proliferation of a small number of opportunistic species, the reduction of distribution range for the key species *Cystoseira*, *Phyllophora* and *Zostera*, but also the reappearance of some considered extinct species.

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