Research and Restoration of the Essential Filters of the Sea (REEFS), Romanian Black Sea Coast *(Angelica-Ionela Paiu, Mihaela-Elena Mirea Cândea, Romulus-Marian Paiu, Cornel Ursache, Laura Boicenco, Florin Timofte, Luminița Lazăr, Camelia Dumitrache)*	"Cercetări Marine" Issue no. 45 Pages 183-194	2015

RESEARCH AND RESTORATION OF THE ESSENTIAL FILTERS OF THE SEA (REEFS), ROMANIAN BLACK SEA COAST

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ABSTRACT

Black Sea, as any other basin, has its ecological problems related to human activities, and because it is almost isolated from World Ocean, the problems are more intense. Massive pre-fertilization of Black Sea, disposal of insufficient purified wastewaters, degradation of bottom algal communities, oxygen deficiency in near – bottom water layers, overfishing and bottom trawling are just a few of these important problems. These negative aspects emphasize the need to begin concrete actions to improve the marine environment. The purpose of construction and placement of 8 artificial reefs in Black Sea Romanian coastal waters is to enhance the fish resources, to improve the hydro biological conditions of marine water, to increase production and biomass in the aquatic ecosystems and to increase self-purifying intensity.

The study of artificial reefs is done in the frame of the project "Research and Restoration of the Essential Filters of the Sea (REEFS)". REEFS project a joint cross-border initiative of five partners from the riparian countries – Bulgaria (Bulgarian Biodiversity Foundation), Ukraine (Odessa Branch of the Institute for Biology of the Southern Seas), Romania (Mare Nostrum NGO), Georgia (Ilia State University) and Turkey (Karadeniz Technical University), within Joint Operational Programme "Black Sea Basin 2007 - 2013", under the European Neighbourhood and Partnership Instrument.

Key-Words: *Black Sea, artificial reefs, polypropilene, REEFS*

AIMS AND BACKGROUND

Being in permanent contact with the shore, the Romanian coastal zone was and is still affected by both human activities and natural phenomena that in the last period occur more frequently. These problems translate in the massive pre-fertilization of Black Sea with nitrogen and phosphorus compounds known as "anthropogenic eutrophication", the disposal of insufficiently purified wastewaters, the degradation of bottom algal communities, the oxygen deficiency in bottom water layers, the overfishing and bottom trawling and the lack of information and economic difficulties. All the researchers that studied Black Sea ecosystems and their evolution found that in the last 20 – 25 years, as the majority of marine coastal ecosystems worldwide, showed some structural and functional changes obviously reflected at the level of associations and benthic biocoenosis. Human activities undertaken in the last three decades of the 20th Century in the marine coast, hydraulic works of large scale (harbor expansion, beach and cliff protection), placing on seashore large industrial targets, and massive discharges of insufficiently purified waters are the main causes that have produced significant changes in marine substrate, and among invertebrates. All these accelerated eutrophication which led to the installation of an acute ecological disequilibrium, characterized by increasing primary production of plankton, appearance of algal bloom phenomena, followed by mortality of benthic fauna and reduction of biodiversity. As the Romanian coastal zone is recognized for its natural biofilters that can effectively contribute to water purification, as marine epibiota, specialists emphasize the fact that these can be used to improve the marine environment in the coastal areas.

Given that the natural recovery of these populations is slow and uncertain, proposing an ecological method to grow the natural biofilter by setting up 8 artificial reefs is welcomed.

Research and Restoration of the Essential Filters of the Sea (REEFS) is a pilot project focused on the scientific research of environmental impact of the artificial reefs in the area of the Black Sea countries. The REEFS Project is a joint cross-border initiative of five partners from the riparian countries – Bulgaria (Bulgarian Biodiversity Foundation), Ukraine (Odessa Branch of the Institute for Biology of the Southern Seas), Romania (Mare Nostrum NGO), Georgia (Ilia State University) and Turkey (Karadeniz Technical University).

Artificial reefs are often described as any human-made structure or equipment deliberately placed in marine environment where that structure does not exist under natural circumstances. The purpose of the construction and underwater placement of these structures is enhancing the fishery resources, increasing populations of all sorts of plants and animal sea life, hydrobiological amelioration and tourist entertainment.

The overall objective of the REEFS Project is to establish a long-term partnership platform for scientific, technical, administrative and awareness raising activities in favor of artificial reefs practice, as a way of active support of the self-restoration of the Black Sea ecosystem.

The total budget of REEFS project is 627,650.12 euro, of which the total amount of the grant is 564 885.10 euro provided by the European Union through the Joint International Programme "Black Sea Basin 2007 - 2013".

MATERIAL AND METHOD

In order to set the station network, there were a few parameters to consider, such as the dynamic of the sea currents in the area, the depth of the water, the natural characteristics of the ecosystem, as well as a wide coverage in order to get conclusive and concise information on the ecosystem. The station network is made up of four stations which are located some 500 meters north, south, east and west of the central point with the coordinates: 44°09’56,3” northern latitude, 28°39’56 eastern longitude (Fig. 1, Table 1).

Fig. 1. Place of the installment, Casino - Constanța

Table 1. Coordinates of stations

Nr. Crt.	Station	Latitude (N)	Longitude (E)
1.	**NORTH**	44°10’07,6”	28°39’47, 2"
2.	**SOUTH**	44°09’74,9”	28°39’91, 8"
3.	**EAST**	44°09’80,6”	28°40’27, 4"
4.	**WEST**	44°10’10,3”	28°40’06, 7"

The water samples and biota were taken by specialized staff from INCDM, with devices of their own – Niskin bottles - and kept in tagged plastic capacities in cooler bags.

The water samples for the determination of the dissolved oxygen were taken in Winkler colorless bottles, with conical top and a close fitting stopper. Each bottle has its own volume engraved, and the takeoff was made carefully in order not to contaminate the sample with oxygen from the atmosphere. The samples were set with specific reagents immediately after the taking off.

Fig. 2. Sampling zooplankton

The zooplankton samples were taken with a Juday net with an internal diameter of 36 centimeters, a filter sieve of 150 µm and a length of 1.5 meters (Fig. 2). The prelevation was made by the vertical towing, with a speed of 0.5 - 1 m/s, of the net in the water mass. In order to ensure a vertical position of the net in the water, 25 kg weights were used. After the prelevation the net was lifted on the ship's deck and was washed with a gentle seawater jet in order to free the organisms stuck in the filter sieve. The cable length was used in order to determine the volume of filtered water.

The bentos samples were quantitavely prelevated with the Van Veen bodengreifer, from a depth of 11 meters.

The quantitative analysis of the fitoplankton was made accordingly to SR EN 15204-2007 (Utermöhl method) and consisted of microalgal sedimentation from a known volume of 10 ml, in sedimentation rooms, followed by the sample analysis with the inverted microscope. The sedimentation (at least 8 hours) was followed by the identification and counting of the fitoplanktonic species, using 40x lenses for small forms (smaller than 15 - 20 µm), and 10x or 20x lenses for bigger forms. The biovolume of the cell was calculated by measuring the fitoplanktonic cells and assimilating them in the correspondent geometric figures (Edler, 1979).

Once brought in the lab, the zooplankton samples were left for sedimentation for at least one week. For the microscopic processing the extra volume in the jar was removed until it reached 100 ml or more, depending on the density of the organisms from the sample. The concentration of the sample was followed by taxonomic sorting under the binocular magnifier and inverted microscope. The sorting was made by extracting a sample of 5 ml from the original sample, and counting the organisms in it. The counting was made in a Bogorozov counting room. The process went on until there were at least 100 specimens from three dominant species. For the rest of the rare organisms or bigger dimension organisms, the samples were fully examined.

After the prelevation of zoobentos, the samples were deposited in plastic bags, set with 4% formaldehyde, tagged and lab processed by washing them with granulometric sieves, eye diameter of 1 mm and 0.5 mm. After washing, every fraction of every sample was separately analysed under the stereomicroscope, manually separating the organisms by main groups of invertebrates from the romanian marine sector: worms, mollusks, crustaceans. For the quantitative analysis, the individuals of each species were counted at the same time with their sorting and identification. The density was measured in individuals per m^2, and the biomass in g/m^2.

RESULTS AND DISCUSSIONS

The main features of the environmental factors in the Romanian coastal zone is the natural variability, marine waters in the marine sector is strongly affected by river input from northwestern basin, the winds, the currents and the succession of seasons. The coastal regions represent distinct ecosystems, whose productivity is influenced by various factors. The production of the continental shelf is linked to river input and climate change (Bodeanu et al., 2002 and 2004), while marine waters are influenced by climatic factors which controll stratification, movement of water (Lehmann, 2008). The nutrients are elements or chemical species involved in the production of phytoplankton of organic material. The current assessment is based on phosphorus, silicon and nitrogen, elements that are efficiently extracted from seawater and are incorporated in cells, tissues, and extracellular structures of marine organisms. Some of them are reclaimed several times in water column while another part settles. Generally, the vertical transport of nutrients is less efficient than the force of gravity, so the concentration increases with depth.

Before the installment of the 8 structures of artificial reefs in Casino area, the concentration of phosphates was 0.33 µM, which fits in the normal parameters of 0.18 to 0.50 µM.

For total phosphorus indicator was recorded values of 1.07 µM, this beeing far below the allowed limit of 100 µM/l for surface waters. The recorded value for silicate contraction was 8.1 μM, which is accordingly with the value allowed for surface waters (N=132) 0,9-55,1μ. The concentration of nitrates in the study area has a value of 1.90 μM, being situated between 0.12 – 15.09 μM, the limits for surfaces waters (N=132). The nitrite concentration was 0.36 μM, being between 0.01 – 1.74 μM, the value accepted for surface waters (N= 132) (Fig. 3).

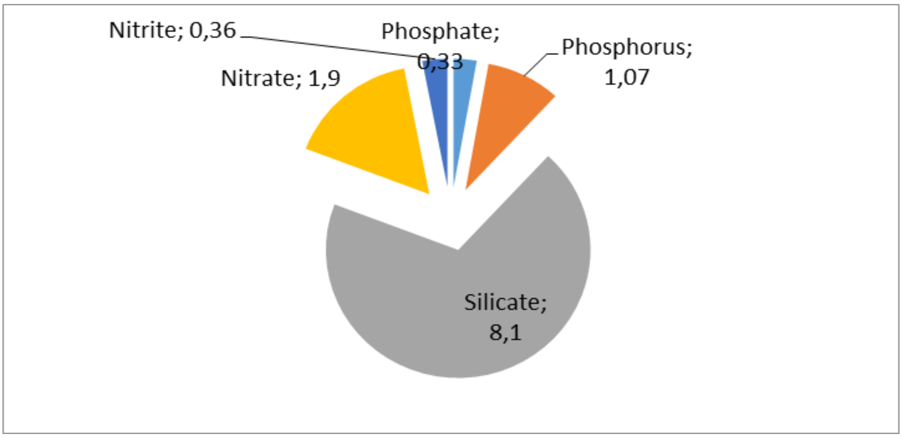

Fig. 3. Concentration of nutrients

Biological parameters

Phytoplankton, which represents all forms of vegetable unicellular from water, is the main primary producer, the base of the food chain pyramid. It represents also the consumer of inorganic and organic nutrients, which enters the sea through river systems and wastewater discharges.

The samples taken before the installment of the artificial reefs showed 14 species of 5 algal groups (*Bacillariophyta*, *Dinoflagellata*, *Cyanophyta*, *Chrysophyta* and *Cryptophyta*) (Table 2).

Table 2. Values of phytoplankton - Casino Constanța station

	DENSITY (cel/l)				BIOMASS (mg/m^3)			
Species	**Diatom**	**Dinoflagellata**	**Other groups**	**Total**	**Diatom**	**Dinoflagellata**	**Other groups**	**Total**
Amphora sp.	880			880	0.27			0.27
Cerataulina pelagica	440			440	1.58			1.58
Chroomonas caudata			7040	7040			4.47	4.47
Cocconeis sp.	880			880	0.27			0.27
Emiliania huxleyi			99440	99440			15.51	15.51
Gymnodinium wulffii		1320		1320		0.19		0.19
Hillea fusiformis			1320	1320			0.46	0.46
Monoraphidium			880	880			0.04	0.04

irregulare								
Neoceratium furca		880		880		48.36		48.36
Peridinea vegetative stages		880		880		16.02		16.02
Prorocentrum compressum		440		440		4.03		4.03
Prorocentrum micans		12320		12320		251.94		251.94
Prorocentrum minimum		3960		3960		4.11		4.11
Pseudosolenia calcar-avis	440			440	25.13			25.13
Total	**2640**	**19800**	**108680**	**131120**	**27.27**	**324.66**	**20.50**	**372.45**

The total biomass registered was 372,45 mg/m^3. Dinoflagellates have the biggest biomass (324.66 mg/m^3), including *Prorocentrum micans* (251.94 mg/m^3), Neoceratium furca (48.36 mg/m^3) (Fig. 4).

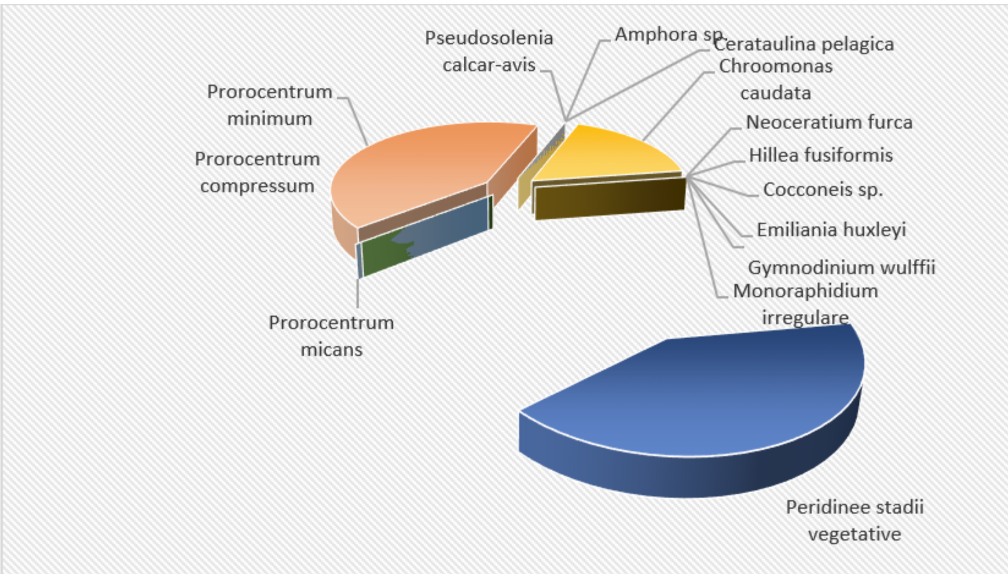

Fig. 4. Total phytoplankton biomass

Qualitative and quantitative structure of zooplankton

After analyzing the samples were identified 10 species that belong to 10 taxonomic groups (Table 3).

Table 3. List of identified species of zooplankton in the study area

Species	Taxonomic groups	
Noctiluca scintilans	Dinoflagellata	*
Acartia clausi	Copepoda (calanids)	*
Oithona similis	Copepoda (cyclopids)	*
Pleopis polyphemoides	Cladocera	*
Polychaeta larva	Polychaeta	*
Bivalvia larva	Bivalvia	*
Decapoda larva zoe	Decapoda	*
Balanus larve	Decapoda (cirripeda)	*
Oikopleura dioica	Apendicularia	*
Parasagitta setosa	Chetognata	*
TOTAL		**10**

From the quantitative perspective, the zooplankton was characterized by rich populations (17271 ind.m^{-3} şi 573 mg.m^{-3}). The dominant component as density was the trophic zooplankton (12371 ind.m^{-3}) and the dominant group was the copepods (*Acartia clausi)* with a density of 10315 ind.m^{-3}. In terms of biomass, dominant were *Noctiluca scintillans* (431 mg.m^{-3}) and *Acartia clausi* (128 mg.m^{-3}).

Zoobenthos

Similar to natural reefs, the artificial reefs can play an important role for the marine ecosystem, creating a support, shelter and feeding for many marine organisms (algae, invertebrates, and fish), enhancing biodiversity and increasing ecological stability in the installed ecosystem, and also increasing the biological productivity of coastal zone. (Steimle F. and all, 1973). Zoobenthos represents the mirror of the phenomena that occur in the water, responding to environmental changes by modifying the qualitative structure (species) and quantitative (number of species, density, biomass) of communities.

Benthic samples were taken from substrate of sand and from artificial reefs structures. Quantitative benthic samples were collected with Van Veen bottom grabs from a depth of 11 m. Epibiota was taken from three points of the reef (surface, center and bottom) with a collector device with 20/20 cm^2. After being sorted at the microscope, then these were manually separated in main groups of invertebrates: worms (polychaeta), shellfish (bivalves), crustaceans (amphipods). The species were identified to species level or group (if applicable) using specific determination keys (Morduhai - Boltovskoi). Density and biomass were expressed in m^2.

The biocenosis in the study area belongs to fine-grained sand, siliceous sand, wherein the fauna is represented by *Lentidium mediterraneum*, the area being considered the most typical with this kind of sand, quite far from the direct influence of the Danube.

The analysis of the specific composition of samples taken before the installment of the artificial reefs led to the identification of 19 species of macrozoobenthos divided in: 9 species of worms (polychaeta) (47%), 6 species of mollusks (32%), 3 species of crustaceans (16%) and other groups (5%). Then, the analysis of the of the specific composition of benthic fauna from sedimentary substrate from the area, after the installment revealed 20 macrozoobenthic species distributed in groups, as 8 species of worms (polychaeta) 40%, 4 species of mollusks (20%), 4 species of crustaceans (20%), 3 species of gastropods (15%) and one group of other species (5%) (Fig. 5).

In both cases, the qualitative structure of benthic fauna was characterized by the bivalve *Lentidium mediterraneum*, followed by other species as *Chamellea gallina*, *Cerastoderma glaucum*, *Cyclope neritea* (first samples) and *Chamellea gallina*, *Anadara kagoshinensis*, *Ecrobia ventrosa*, in case of the second round of samples.

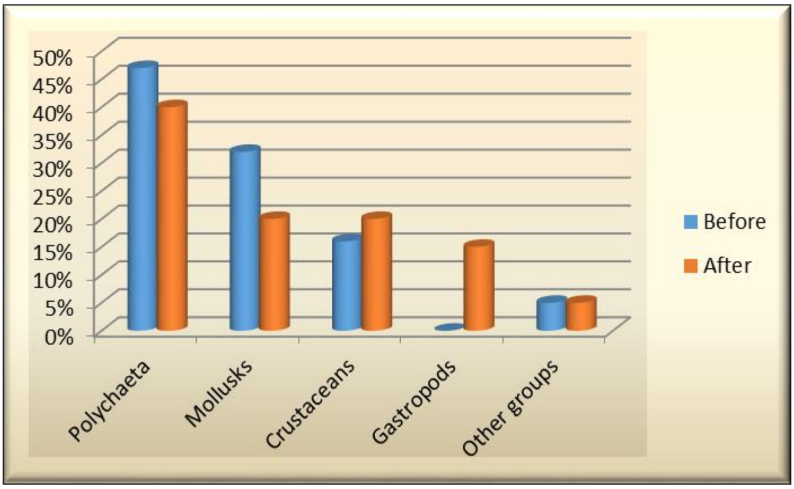

Fig. 5. The percentage distribution of the main groups of macrozoobenthic invertebrates in Casino Constanța

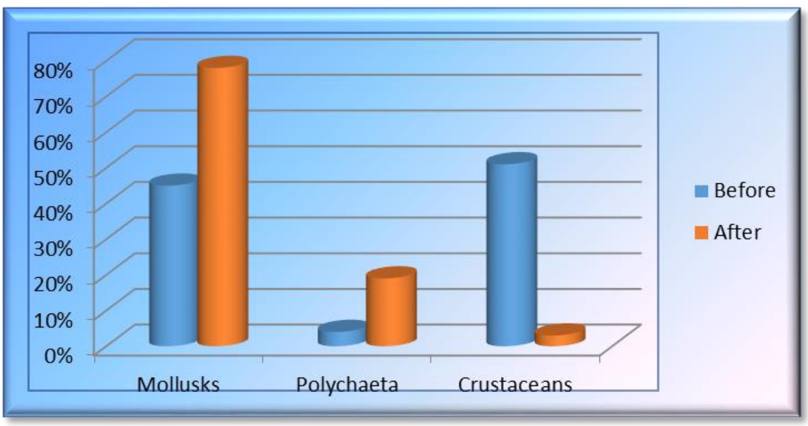

Fig. 5. Distribution of the main groups of benthic invertebrates

Before the installment, the density of the macrozoobenthos was dominated by crustaceans with 51% of total density, followed by mollusks 45% and polychaeta 4%. The numerical dominance belonged to crustaceans, to amphipod *Ampelisca*, species with wide ecological valence.

In terms of quantity, after the installment, the densities of the macrozoobenthos were dominated by mollusks, 78% of the total density, followed by polychaeta with 19% and crustaceans with 3% (Fig. 5). Regarding the numerical dominance of the mollusks fauna, the *Lentidium mediterraneum* was dominant.

Qualitative composition of epibiota developed on artificial reefs

Analysis of the qualitative composition of the epibiota taken after a few weeks after the immersion from artificial reefs were identified 8 types of organisms: 4 are sessile (*Balanus*, *Mytilus galloprovincialis*, *Membranipora*, macrophyte algae) and 4 are vagile (*Neanthes succinea, Microdeutopus gryllotalpa, Pleonexes gammaroides, Copepods).*

Epibiota developed in this period is dominated, quatitative, by the crustacean *Balanus improvisus. Mytilus galloprovincialis* is a species with a high degree of euryhaline, being very active in forming epibiota. The mussules replace the balanus after 1-2 years and give high biomass mostly on submerged stationary objects. This can be attributed to the fact that Mytilus larvae is fixing harder then Balanus in conditions of waves and strong currents and due to their slower rate of growth.

Among macrophyte algae, first as frequency are the green algae species and here we mention *Enteromorpha*, *Cladophora* which have a wide adaptability to the environmental conditions (salinity and pollution).

Along with sessile organisms present in epibiota from artificial reefs were identified and vagile organisms, the mst important group being the polychaeta. In a more advanced stage appear and other groups as: copepods, amphipods (*Microdeutopus gryllotalpa*, *Pleonexes gammaroides).*

CONCLUSIONS

The analysis of the data obtained from the place of the installment of the 8 structures of artificial reefs revealed that the area corresponds spatially to fine-grained sand biota, with *Lentidium mediterraneum*, as dominant. Also, the analysis of the specific composition of the macrozoobenthic fauna showed the presence of 20 macrozoobenthic species and in terms of quantity, the mollusks dominate the density of macrozoobenthos with 78%, being followed by polychaeta, 19% and crustaceans 3%. Then, the epibiota developed on reefs consists of 2 basic sessile species, crustacean *Balanus improvisus* and *Mytilus galloprovincialis* bivalva, these having a great capacity to fill any immersed substrate, are resistant to large variations of environmental factors, have a high fecundity and high rate of growth. Regarding the vagile fauna, it was represented by three macrobenthic species, *Neanthes succinea* and *Microdeutopus gryllotalpa*, *Pleonexes gammaroides.*

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