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Sigma energija d.o.o.

Družmirje 20, 3325 Šoštanj, Slovenia

Subject: Report on the post monitoring of the sea ecosystem – seabed after deploying the WEC in the aquatory of Bar, Montenegro

Dear Sir/Madam,

as per our Agreement on the post monitoring of the sea ecosystem – seabed after deploying the WEC in the territorial waters of Bar signed by Sigma energija d.o.o., legally represented by the General Manager David Volk, and the University of Montenegro, Institute of Marine Biology, we deliver the report in accordance with the deadlines arranged with the client.

Yours faithfully,

Director

Dr. Aleksandar Joksimović

Kotor, December 2022

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Introduction

Researching the macrozoobenthos where the WEC is located has been carried out in line with the Agreement. Extensive biological research on biodiversity was carried out during the zero-state analysis. The research included plankton as well as nekton analyses. The sampling of the seawater was done in close proximity to the deployment location to analyse the plankton component (phytoplankton, zooplankton, and ichthyoplankton), chlorophyll a as well as the water's microbiological quality. The analysis of phytobenthos, macrozoobenthos, and infauna has been carried out with a remotely operated vehicle (ROV) on a surface of 50x50m and sediment samples while the ichthyofauna analysis has been carried out in the aquatory in front of the Port of Bar.

Given that this is a one-time survey that needs to show the impact of the WEC on living organisms at the location and its close proximity, we have agreed to investigate benthic organisms that actually suffer the most and are directly impacted by all changes at the location.

The zero-state (base-study) analysis has shown that the communities on the seabed at the site show no presence of algae. One of the reasons is the moveable seabed that does not allow these sessile organisms to attach, and the other is a depth of almost 30m and a small amount of light reaching the seabed, thus creating unfavourable conditions for the development of the plant component.

Based on the listed facts, in our research of benthos components we have analysed the state of the macrozoobenthos from a qualitative perspective as well as quantitatively through sediment samples.

Biological and morphological characteristics of the seabed

The seabed is an extremely important structure in the development of a marine ecosystem. The type of organisms or the seabed community that will develop depends on the seabed configuration and material. Two characteristics of the seabed are important from a bio-ecological point of view: the relief and the physical structure. We distinguish among the following types of seabed: the foreshore, continental shelf, continental slope, and deep abyssal and hadal zones.

The foreshore is a narrow strip of the seabed within the tidal range, thus amphibian in nature because it is underwater at high tide and above water level at low tide. This stripe is very much exposed to the mechanical effects of seawater and is characterised by periodical changes of the physical and chemical environmental conditions.

Deeper down, the foreshore continues into the continental shelf extending up to the global average depth of up to 200m. The shelf is basically the continent margin.

We distinguish between three basic seabed types in terms of physical structure: rocky, sandy, and muddy.

Rocky seabed is present in areas where fine sand and mud cannot be deposited due to the increased water mass dynamics (waves and currents) as well as due to a larger slope. Rocky seabed is closer or next to the shore but do not extend beyond the shelf margin. In a biological sense, a rocky bottom is every hard surface – natural or artificial (gates, buoys, ship remains, driftwood, concrete blocks etc.).

Affected by the physical and chemical effects of water (rivers, torrents, waves, streams, in addition to the effects of living things), rocky seabed breaks apart into gravel, sand, and mud, and that material is then deposited onto the seabed close to or further from the shore, depending on the material granulation and the power of the water masses.

Sandy bottom consists of grains not smaller than 1/10 mm on average, are slightly aggregated and mostly silicate in nature. Deposition of such sand occurs close to the shore, in shallow waters, which is why sandy seabed does not extend beyond the shelf boundary. In protected areas (protected from stronger water movements), sand deposits may occur on the shore itself.

The transition between the rocky and sandy seabed is gravel and shelly bottom. Gravel has an average diameter of 3mm, while shelly bottom is of organic origin (remains of Mollusca, Echinodermata, Crustacea, calcareous algae, etc.).

The muddy bottom covers most of the seabed and consists of particles smaller than 1/10 mm. It is found in areas where the dynamics of water masses is reduced to a minimum. Mud particles are of dual origin: terrigenous (terrestrial) and pelagic. The terrigenous elements of mud are transported to the sea by rivers, torrents and winds, they have different chemical compositions, concentrations, external appearance and colour.

Living organisms on the seabed represent the benthic zone which extends from the shore to the highest depths. In the vertical distribution of benthic communities, the basic term is a step or floor, while their set constitutes a system. With increasing depths, the seabed system distinguishes between several steps as described below.

- The supralittoral step is the one inhabited by organisms that tolerate and require constant emersion (surfacing). It is therefore a stage of seawater wetting and is located above the spring high tide line.

- The mediolittoral step is the zone of high tides and low tides, i.e. the zone reached by high water during high tide and the lower limit of the water level during low tide. The organisms that live there require alternation of emersion and immersion.

- The infralittoral step is bounded by the lower limit of the low tide towards the mediolittoral step, and towards deeper waters with the zone of marine phanerogams and photophilic algae – the lower limit of these communities. For the Mediterranean Sea, these are depths of about 20m.

- The circalittoral step extends from the lower boundary of marine phanerogams (or photophilic algae) up to the border depth compatible with the vegetation of algae most tolerant of low light, i.e. the most sciophilic. The set of previously defined four steps, whose names contain the suffix littoral (supra, medio, infra and circa), makes up the littoral or coastal system or given the presence of benthic chlorophyll plants – the phytal system.

Deeper down (to the greatest depths) we have the bathyal, abyssal and hadal zones.

General characteristics of the coastline, littoral or phytal system

The merit of the littoral or phytal system is the richness of plant and animal species. It contains all benthic autotrophic species. Even for the animal benthic species, we can say that 99% of all known species occupy the littoral system.

The diversity of life in this layer depends on suitable ecological conditions. The phytal system has the most diverse temperature conditions. All thermal demands can be met there. Some warm, stenothermal species will localise in tropic regions, for example, while some cold stenothermal species live in the polar regions. Between these two extreme regions, there is space for all kinds of ecological requirements in terms of temperature. The phytal system has the most diverse substrate conditions. Various rocks, pebbles, sands and silts are substrates that each have their own characteristic community for a certain seabed. This system is characterised by the most diverse sources of organic matter necessary to sustain the animals.

Despite all the mentioned optimal conditions, benthic life in the phytal system is not without problems. Namely, it is the zone of strong currents, which for species with indirect development creates the danger of their larvae being carried away to distant zones, where the environmental conditions are not favourable for their metamorphosis. This is the zone of the most pronounced variation of physical and chemical parameters, especially temperature and salinity, so these areas are inhabited by eurythermal and euryhaline species - species that tolerate large amplitudes in temperature and salinity, i.e. types of wide ecological valence. Anthropogenic impacts are the most pronounced in this zone.

In the bottom or benthic zone, there are numerous types of organisms that are adapted to the environmental conditions and have created some of their own adaptations in relation to them. The division of organisms that live on the seabed is as follows:

- Sessile ie. forms that are permanently attached to some solid substrate, including many hydroid polyps, all sponges, bryozoans, ascidians, many shells, snails, polychaetes, cirripedia, and others. The manner of fixation and the morphology of sessile forms are endlessly different. Important factors that affect fixation are: the size of the fixation surface and its ratio to the total surface, shape, dimension and strength.

- Rooted forms (pivots) are immovable forms characteristic of a movable substrate, which, instead of being spread over the surface of the substrate, are drawn into it by root-like appendages. These include numerous actinidia, penatularia, and many species of polychaetes.

- Sedentaries or sedentary forms are not fixed, but their movement is of very small amplitude (1-10 m). This is where most snails and echinoderms belong.

- Vagile forms are benthic forms that have a greater amplitude of movement than the previous category (decapod crustaceans, many snails, cephalopods, some fish).

- Free forms. There is a small number of benthic forms that are truly free in the sense of the mentioned distribution, i.e. mobility in relation to the substrate.

Zoobenthos analysis

Materials and methods

The study site, where the zoobenthos research was conducted, is located at Cape Volujica (Figure 1). Sampling was done by the method of autonomous diving.

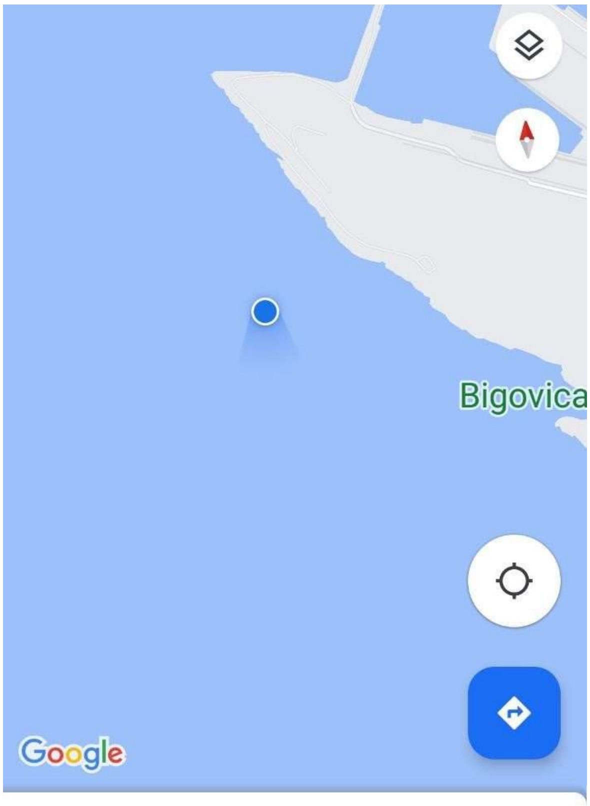

Figure 1: Research area

During fieldwork, 3 sediment samples were taken for infauna analysis at a depth of about 30m. The distance between the points where the sediment was sampled is 50m. The substrate at the sampling site is soft and mobile and is mainly represented by silty-sandy sediment with admixtures of decayed leaves of the marine phanerogams *Posidonia oceanica*, which is not present at the sampling site. Under the influence of sea currents, parts of decayed leaves reached the research site.

Sediment samples were sieved through a 1-mm sieve and the collected material was preserved in 70% ethanol, and its taxonomic affiliation was determined with the help of a stereomicroscope in the Laboratory for Benthos and Marine Protection, Institute of Marine Biology (Figure 2).

The results obtained from the processing of sediment samples were processed with the Primer statistical software to see values such as the number of species ("S"), number of specimens ("N"), the Margalef's index ("d"), evenness index of species distribution (J'), Shannon-Wiener index ("H") and dominance index ("λ").

Figure 2: Stereomicroscopes for laboratory processing of samples

Fouling sampling included the collection of living organisms that developed on the submerged parts of the WEC. Immediately after sampling, the material was preserved in 70% ethanol and further analysed in laboratory conditions.

During field research, photo documentation is created, which represents the least invasive method, and serves as a complement to sample analysis because it is used for species that can be recognised *in situ*. Epifauna research included determining the presence of macrozoobenthos species on the surface of the substrate.

Analysis results

Descending to the seabed in order to observe the condition of the epifauna and take sediment samples for the infauna showed that the site in question is located in an area where soft bottoms, i.e. mobile substrates, are represented (Figure 3). Such substrates are mainly composed of different fractions of sand and silt and as a substrate type they are very unfavourable for the development of benthic communities. Even during the analysis of the base study, it was established that the benthic communities in this locality were reduced, i.e. minimally developed. The lack of solid substrate affects the absence of sessile species. These organisms need a solid base to which they can attach themselves and continue their growth and development. Soft substrates are therefore characterized by the complete absence of sessile and poorly mobile forms. Epifauna analysis showed a complete absence of macrozoobenthic organisms.

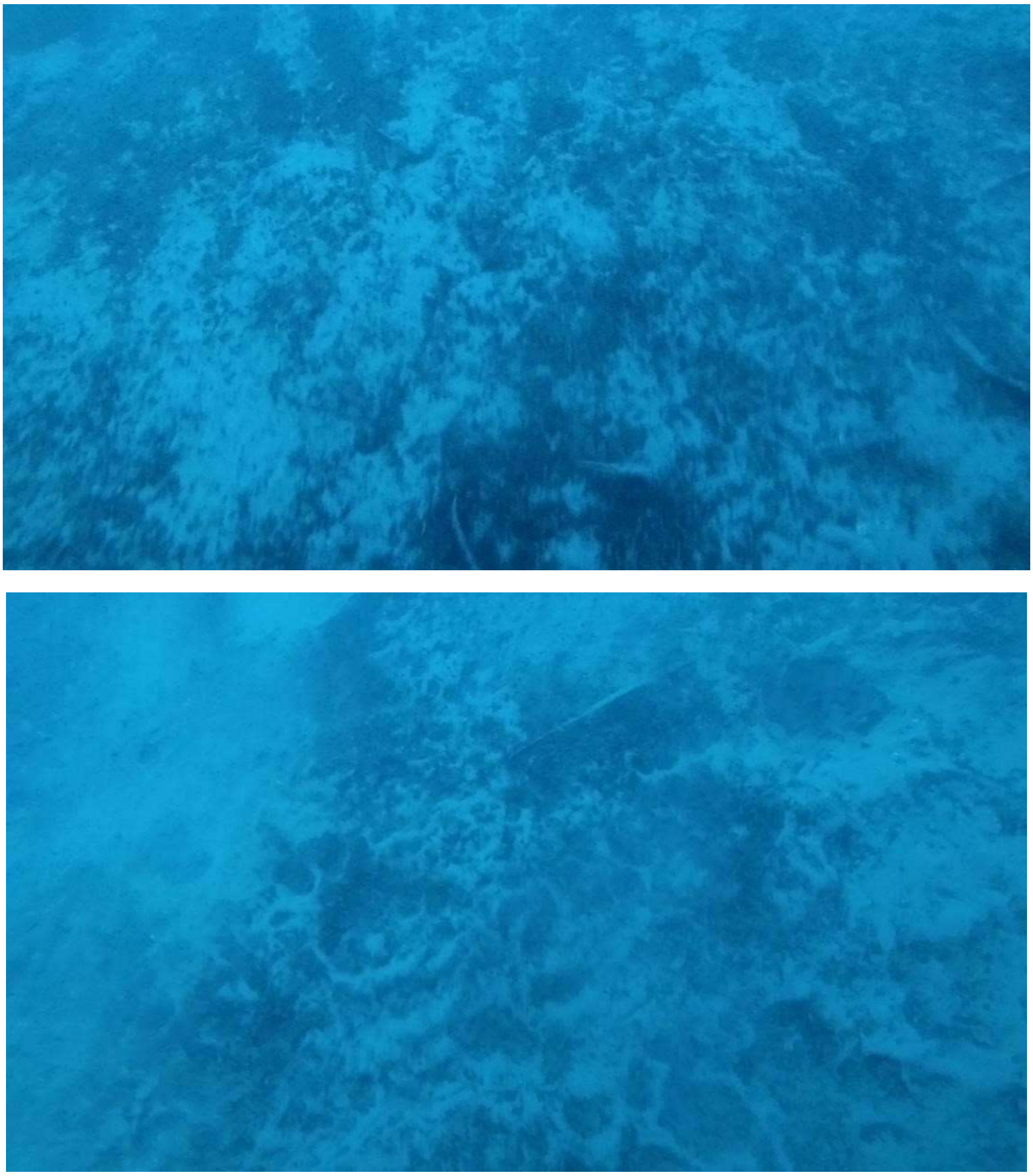

Figure 3. The surface of the seabed consists of a soft substrate composed of sand and fine silt

The infauna analysis included the analysis of 3 sediment samples that were collected at a distance of about 50 meters from one another. Based on the processing of the collected material, the presence of 43 species was determined (Table 1). Most of the identified species were from the group of molluscs, which includes bivalves and gastropods (36 species), from the group of bryozoans there were 5 species, 1 species of annelids, and one species of echinoderms. All identified species are typical of the substrate type and depth at which they were sampled (Figure 4).

Table 1: List of infauna species at the investigated locations

Species	Point 1	Point 2	Point 3
Acanthocardia paucicostata	3	2	1
Anomia ephippium		2	
Atlanta sp.			1
Bittium lacteum		1	
Bittium reticulatum		3	
Calliostoma sp.	1		3
Calyptraea chinensis			2
Celleporina lucida	1		
Cerithiopsis tubercularis		2	3
Ditrupa arietina	5		2
Dosinia lupines			2
Emarginula fissure			1
Entalophoroecia robusta	1		
Eulima glabra	2		
Euspira catena	1		1
Euspira sp.	1		
Frondipora verrucosa	2	2	3
Haminoea navicula		1	
Laevicardium crassum	1		2
Mangelia tenuicosta	1		
Mimachlamys varia	2		1
Moerella donacina	2		2
Moerella pulchella			4
Myrtea spinifera		2	2
Ophiura albida			1
Papillicardium papillosum	2	3	3
Patinella radiate	1		
Phaxas pellucidus			3
Philine quadripartita	1		
Pitar rudis	3	2	2
Retusa truncatula	1		
Ringicula conformis	1		
Ringicula cuspidata	1		
Rissoa sp.	2	3	
Schizobrachiella sanguinea	1		
Schizomavella sp.			1
Thracia distorta			3
Timoclea ovate	4		5
Tritia lima	2	3	
Tritia reticulata	2	2	4
Turbonilla sp.		2	
Turritellinella tricarinata	2		
Varicorbula gibba			3

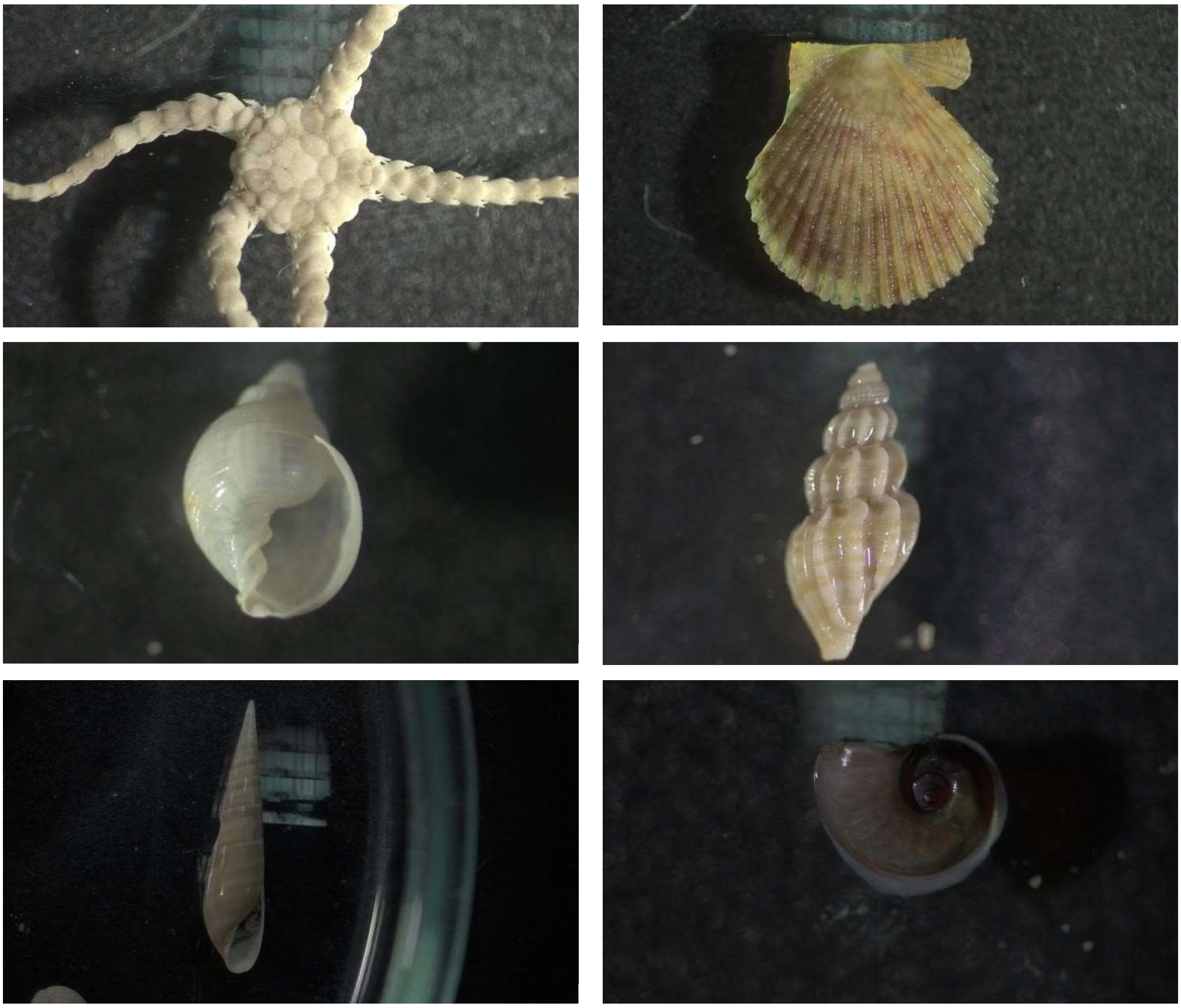

Figure 4: Species from the infauna sample

The application of statistical data processing shows that the highest number of species was recorded at Point 1 (26 species), followed by Point 3 (24 species) and Point 2 with the fewest species (14). Based on the number of specimens, Point 3 was the richest (55 specimens), while point 1 had 46 specimens, and the smallest number of specimens was in the sample from Point 2 (30 specimens). The analysis of the sample diversity index (Shannon-Wiener index) indicates that the species diversity in the samples was the highest in the sample from Point 1, while the diversity in the other samples was also at a satisfactory level. In terms of the uniformity of the number of species per sample (“J'”), the situation is uniform, ie the results show that all identified species had a similar specimen count (Table 2).

Table 2: Diversity index values (S – number of species, N – number of specimens, d – Margalef's index, J' – evenness index of species distribution*, H' – Shannon-Wiener index, λ – dominance index)*

	S	N	d	J'	H'(loge)	1-Lambda'
Point 1	26	46	6.529731	0.957193	3.118626	0.970048
Point 2	14	30	3.822183	0.982197	2.592074	0.954023
Point 3	24	55	5.739478	0.963945	3.06347	0.96633

Based on the results of statistical processing, it can be concluded that the infauna at the sampling location is relatively well developed.

The identification of fouling material has shown that the degree of development of fouling communities is very different on objects that are permanently submerged in water. The reason for the differences may be the material of the submerged objects (metal, canvas, plastic) as well as the different lengths of time of stay underwater (it is considered that not all objects were submerged at the same time) (Figure 5). Fouling communities use solid substrates for their development, to which they attach themselves and thus resist the movement of water, which tends to tear them off with its force. The degree of development and diversity of these communities depends on the time given to the communities for development as well as on the individuals, gametes, and diversity of the surrounding living world.

Figure 5 Different structures show different stages of fouling

The analysis of fouling samples showed the presence of 17 species of macrozoobenthos (Table 3). Of the total recorded species, the largest number are species from the group of annelids or worms, of which there were 5, there were 4 species of tunicates, bryozoans were represented by 3 species, cnidarians and molluscs (Mollusca) with 2 species each, and crustaceans (arthropods) were represented by one species.

The general state of fouling communities is in the initial stage of development. A smaller number of species was noticeable, while the density of their populations was very high. In the following period, as the fouling communities become more complex, there will be changes in the structure so that the number of species present will increase and the number of their populations will decrease. All recorded species are typical filter feeders.

Table 3: Fauna species recorded in the fouling samples

Phylum	***Species***
Cnidaria	*Eudendrium sp*
	Stylactis inermis
Annelida	*Protula sp.*
	Janua heterostropha
	Spirobranchus sp.
	Filograna
	Serpula vermicularis
Mollusca	*Patella caerulea*
	Anomia ephippium
Crustacean	*Perforatus perforates*
Briozoa	*Bugula neritina* *
	Schizobrachiella sanquinea
	Bugula plumosa
Tunicate	*Didemnum cf. fulgens*
	Styela plicata *
	Botrylloides leachii
	Ascidia mentula

*Introduced species

Figure 6: Dominant fauna species in the fouling

Based on the observation of the state of the infauna in the sediment of the seabed, it can be concluded that it is relatively well developed. The results of the research show that the epifauna is insufficiently developed, which is typical for mobile, soft bottoms. The situation was the same during the analysis of the base study. The fouling communities on the submerged parts of the WEC are in the initial stage of development. There were no protected (endangered) species among the identified species in the vegetation, while two introduced species (*B. neritina* and *S. plicata*) were recorded, which have been known to inhabit the Montenegrin coast since before.

The results of macrozoobenthos research at the location show that the activity carried out does not have a negative impact on its diversity.

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