Journal of *Marine Science and Engineering*

MDPI

Article

Effect of Long-Term Immersion in Low-Salinity Seawater on Epoxy Resin Composites Filled with Marine Secondary Raw Materials

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Abstract

This research explores the potential introduction of marine waste-derived biological fillers within bio-epoxy matrices to mitigate the environmental impact of traditional materials, like fiberglass, in boat construction. However, this raises concerns about biofouling and degradation, issues that have not been extensively investigated in composites, especially over a time frame representative of issues that could arise during service. Although protective solutions like biocides and specific coatings exist, degradation remains challenging when attempting to use eco-friendly natural fillers. This study specifically integrates various biological fillers, namely ceramics (mussel, oyster, clam powder) or ligno-cellulosic (i.e., *Posidonia oceanica* fibers) into epoxy for use in some boat components (bench seats for the bridge deck), aiming to evaluate the biofouling process under extreme (or decommissioning) conditions. In itself, epoxy does represent an ideal enclosing matrix for biomass waste, which ideally needs to be introduced in significant amounts. The development of biofouling in the specific context of Kotor's Bay, Montenegro, for a duration of six months, and relevant composite degradation were examined. In particular, three situations were reproduced by positioning the samples in a harbor environment: (i) on the bottom of the sea (2 m. depth), (ii) immersed just below the surface (0.5 m. depth), and (iii) on the splashing surface (pier). The concerns identified appear generally limited in the case of the envisaged application, despite some significant wear effect in the case of the samples containing Posidonia. However, this study also offers information and caveats in terms of more ambitious prospective applications (e.g., the boat hull structure).

Keywords: bio-based; sea-derived materials; mollusk shell powders; *Posidonia oceanica* fibers; biofouling

Academic Editor: Weicheng Cui

Received: 16 August 2025
Revised: 18 September 2025
Accepted: 11 October 2025
Published: 16 October 2025

Citation: Vicentini, G.; Santulli, C.; Mattiello, S.; Matassa, R.; Nikolić, D.; Petovic, S.; Pesic, A.; Gagic, R.; Felici, A.; Fragassa, C. Effect of Long-Term Immersion in Low-Salinity Seawater on Epoxy Resin Composites Filled with Marine Secondary Raw Materials. *J. Mar. Sci. Eng.* **2025**, *13*, 1985. https://doi.org/10.3390/jmse13101985

1. Introduction

Sustainable alternatives that aim to reduce as much as possible the environmental impact of traditional materials, such as fiberglass, are increasingly being explored for

the construction of boat components [1]. A simple but normally effective approach is to include in the host material some waste, which hopefully exerts a non-negative action on the material properties, while at the same time resulting in a reduction in resource depletion during its production. One of the most appropriate host materials, both for stability and the relative facility of curing to a hard structure, is constituted by epoxy resins, which, not surprisingly, have been considered in this role, e.g., for the inclusion of construction and demolition waste (CDW) [2]. Of course, the intention is that the waste introduced, especially in the case of ceramics, could result in some improvement to the resin's properties [3].

In terms of filler, the first and most obvious possibility is to use thermoplastic particles, which are not economically convenient to recycle, namely due to their ineffective collection, resulting in a mixture of polymers, such as poly(ethylene terephthalate) (PET), or expanded polystyrene [4]. This suggestion stems from the low cost of their use in the construction industry, often in the form of a so-called "plasmix": such a filler does not harm the properties of the host material, although it does not increase its quality, creating only limited value [5].

However, in general terms, from a circular economy perspective, it would be recommendable, whenever possible, to incorporate into the host material refuse from within the same value chain (e.g., marine waste into boatbuilding). Applying this concept, for instance, to the partial substitution of fiberglass in boats, implies that materials more prone to being affected by seawater exposure are introduced into the structure, which then leads to the potential development of biofouling damage. Obviously, it is not expected that these materials will fully replace fiberglass, especially because of the difficulty in obtaining a sufficiently high tensile strength in the absence of elongated fillers. However, it is very likely that their introduction into conventional composites will increase their sustainability. This issue has been investigated in composites, although not extensively, concentrating in particular on the analysis of biofilm, where algae gradually develop on the surface [6], followed by the formation of macrofoulants, particularly constituted by barnacles [7]. In this respect, several protective solutions have also been proposed, including biocides [8] and specific coatings, such as paints [9]. More generally, the growing demand for eco-friendly composites has led to the wide-ranging exploration of natural fillers, such as ligno-cellulosic fibers and ceramic waste, in regard to their application in the marine context [10,11]. More specifically, difficulties are encountered in balancing the mechanical properties, costs, and degradation of such composites during service, and, hence, the occurrence of biofouling [12]. On the other hand, epoxy resins have been proposed as being protective against the effect of saltwater e.g., on geopolymers [13]. Also, the use of bio-based epoxies [14], partially based on a variety of vegetable oils [15], could, as in other fields, increase the sustainability of marine structures with respect to the traditional fiberglass used in this sector [16]. Of course, the question of biological colonization of this type of structures remains unanswered. Biodeterioration is a phenomenon also present in epoxies, though with less evidence than in natural materials, and has received some attention regarding exposure to open air, even in not particularly aggressive conditions, such as in [17]. Beyond this, over time, marine organisms can promote resin erosion, considerably reducing its resistance both to wear propagation and in a wider mechanical sense [18].

Due to the growing demand for the use of sufficiently performing materials from secondary raw materials suitable for marine environments, the present research focuses on the integration of various biological filler materials derived from marine waste into epoxy matrices [19]. The aim of this exploratory research is to intensify the conditions of use in order to evaluate the durability of the materials, providing insights on how to retain an acceptable performance while promoting sustainability (as tentatively investigated

in [20]). The fabrication of composites including this type of waste intended for use in boat components is currently under investigation and will be the focus of further work.

More specifically, in this work, we decided to investigate the effects of long-term immersion of polymeric composites filled with marine secondary raw materials. For purpose, both traditional and bio-based epoxy resins were used, filled with ceramic powder from ground mollusk shells (i.e., mussel, oyster, clam) and/or ligno-cellulosic short not-oriented fibers (i.e., backshore-beached, washed, and dried *Posidonia oceanica* (PO)) [21]. The amount of filler selected for production was decided according to the results obtained in previous studies, starting from [22].

Exposure to seawater was carried out under real field conditions at three different immersion levels (or sites). In particular, Site 1 involved immersion in deeper water at a depth of 2 m, Site 2 was just below the surface at a 0.5 m depth, and Site 3 was in the splashing zone on the pier, and therefore, mainly affected by atmospheric conditions. The last site can be considered the most normal situation of use for the structure, while the other sites represent situations with higher wear and degradation, thus ideally representing simulations of accelerated aging.

2. Materials and Methods

2.1. Raw Materials

Mussel, oyster, and clam shells were obtained from food waste in the Adriatic Sea region, where they were otherwise disposed of as refuse. The shells were ground in a mill to obtain powder with particle sizes between 63 and 88 microns. X-ray diffraction analysis highlighted the presence of different amounts of the two polymorphs of calcium carbonate, calcite and aragonite, as reported in Table 1 and confirmed by previous investigations on mussel, clam, and oyster powders from the same origin [23]. Moreover, the presence of some silica has been revealed in oyster shell fragments, which is suggested to be due to its higher complexity [24].

Table 1. Contents of calcium carbonate polymorphs in seashells [23].

Seashell	Calcite (%)	Aragonite (%)
Mussel (*Mytilus galloprovincialis*)	75.7	24.3
Oyster (*Ostrea edulis*)	98.5	1.5
Clam (*Ruditapes decussatus*)	1.1	98.9

Posidonia oceanica (PO) was collected as dead seagrass stranded on the backshore of Boka Kotorska (Kotor's Bay), Montenegro, where it would otherwise have been gathered and incinerated as waste [22]. After a preliminary process of washing and drying, PO fibers were extracted manually from the egagropili (sea balls) and cut into stretches of length between 5 and 10 mm. These fibers, after careful washing to remove silica and salt, were purposely cut down to 10 mm to be sized into ribbon-like filaments to prevent re-agglomeration into globular structures, which would particularly hinder mixing with resin. To observe their structures, SEM analysis based on secondary electrons with a 1024 × 768 pixel resolution, with 2788× and 1121× magnification, was used. SEM analyses were carried out using Field Emission Scanning Electron Microscopy equipped with a backscattered detector (BSD) (FE-SEM, Sigma Family, Zeiss, Jena, Germany) to obtain high-quality microphotographs.

2.2. Composites

The production of the composites considered in this study has been previously addressed in [23], where untreated lignocellulosic fillers, namely *Posidonia oceanica* fibers, were incorporated in lengths between 3 and 5 mm, along with mollusk shells (oysters, clams, and mussels) ground into powder with particle dimensions between 63 and 88 microns.

Two commercial epoxy casting resins were used:

- A conventional epoxy resin, code RP 026 UV, with IPE 743 hardener, by Trias Chem (Polo di Torrile, Parma, Italy);
- A partially (30%) bio-based resin, code Super Sap CCR (Clear Casting Resin), with CCF (Clear Casting Fast) hardener, by Entropy Resins, Genoa, Italy.

Composites were produced by a manual casting process with slow amalgamation of the fillers to avoid clustering, followed by curing at a temperature of 25 ± 2 °C under continuous monitoring. From the set of composite beams prepared for three-point flexural testing, according to the reference standard ASTM D790-17 [25], that is, with a total length 150 (±2) mm, width 15 (±0.5) mm, and a target thickness equal to 3.2 mm, samples for marine conditioning were removed to assess biofouling. All the categories of samples are listed in Table 2. To observe the behavior of the resin in water, two reference specimens were made with epoxy resin (category A) and eco-epoxy resin (BA). Three epoxy resins loaded with *Posidonia oceanica* fibers at 5% (R), 10% (S), and 15% (T) of the volume were made to evaluate the possibility of fabrication with different levels of sea waste. The geometries selected for the study were determined by the need to create the simple carousel structure for immersion, as depicted in Figure 1. The study focused only the evaluation of fouling at the final time point, that is, after six months. Although it is well known that other sample geometries and configurations might appear more suitable for the study of biofouling, the need for a compact yet simple structure that is able to withstand the simultaneous immersion of a large number of specimens for a long time led to this choice. In fact, the carousel structure appeared intact at the time of extraction after the immersion period. This is promising for future performance in more experiments for even for longer time periods, in the harbor context.

All other specimens were produced using 15% of different fillers with bio-epoxy: mussels (BB), oysters (BC), clams (BD), and *Posidonia oceanica* (BE). It was suggested that the employed resin, as is normally the case for bio-epoxies, absorbs minimal amounts of water, in line with the total hydrophobicity of epoxy [26]. The preparation process involved the collection of mollusk shells from the Kotor's Bay area, followed by a careful washing phase and removal of protein residues. They were then ground with a jaw mill and finally sieved, obtaining a particle size between 63 and 88 microns. For each type of specimen, three samples were produced to be immersed at the three selected sites. Table 2 summarizes the categories of samples produced for research purposes.

Table 2. Samples categories.

Category	Resin	Filler	Amount (%)
A	Epoxy	-	-
R	Epoxy	Posidonia	5
S	Epoxy	Posidonia	10
T	Epoxy	Posidonia	15
BA	Eco-Epoxy	-	-
BB	Eco-Epoxy	Mussels	15
BC	Eco-Epoxy	Oyster	15
BD	Eco-Epoxy	Clam	15
BE	Eco-Epoxy	Posidonia	15

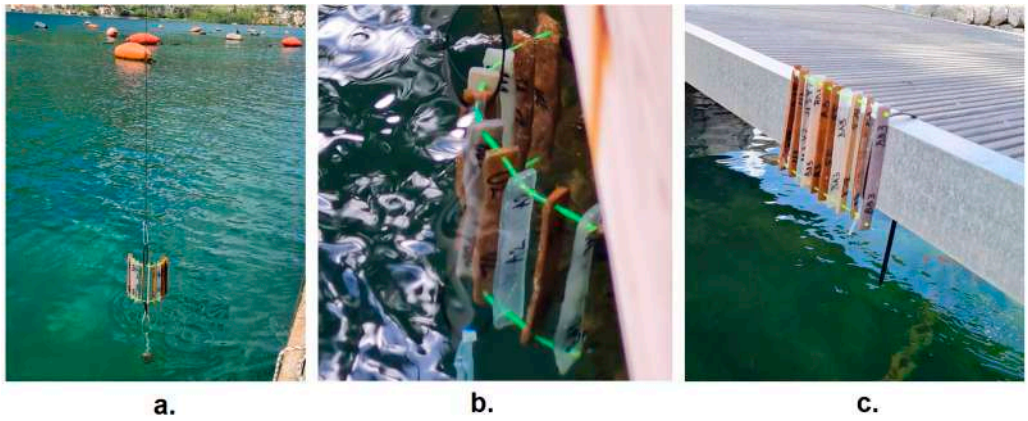


Figure 1. Sample conditioning. (**a**) Towards the bottom of the sea; (**b**) immersion below the surface; (**c**) on the pier.

To construct the carousel structure for immersion, 2 mm-diameter holes were drilled close to the ends of the samples in order to tie them with tubular filaments. The samples were anchored with cable ties at the three selected sites for six months, from the beginning of May to the end of October in front of the Institute of Marine Biology in Kotor's Bay. In At Site 1 and Site 2, the samples were placed respectively at 2 and 0.5 m depths in the water, while at Site 3, the samples were placed in the splashing zone on the pier, where they were mostly subjected to atmospheric conditions (Table 3 and Figure 1). Site 1 and Site 2 were intended to represent intensification of the conditions compared to real application conditions, which were represented by Site 3, to understand what would occur in unexpectedly harsher situations to which the boat could be exposed. Harsher conditions could also offer insight into the accelerated aging procedure of these materials, extending the significance of what has been studied in the six-month period.

Table 3. Samples' locations.

Samples	Site	Location
A1, R1, S1, T1, BA1, BB1, BC1, BD1, BE1	1	On the bottom of the sea
A2, R2, S2, T2, BA2, BB2, BC2, BD2, BE2	2	Immersed just below the surface
A3, R3, S3, T3, BA3, BB3, BC3, BD3, BE3	3	On the pier

2.3. Environmental Conditions

For six months, from May 2024 to the end of October 2024, temperature and salinity data were recorded using data loggers measuring atmospheric temperature (T_{atm} in °C), sea surface temperature (T_{sea} in °C), and water salinity (S_K in PSU) at the following locations:

- At open sea (coordinates 42.46°, 17.29°), approximately at 20 nautical miles by the mouth of the bay;
- Inside Kotor's Bay, in front of the Institute of Marine Biology, at the immersion site of the specimens (coordinates 42.43604°, 18.76342°).

Data were recorded at intervals of 4 h for a total of 6 daily recordings.

During the period of exposure, the thermo-hygrometric conditions were recorded using two data loggers: the VuLink (Watec, Veggiano (PD), Italy), a cellular and satellite telemetry device for remote water monitoring, and the Aqua TROLL 100 (Veggiano (PD), Italy), which measures and records the conductivity and temperature of the water. The direct correlation of data from thermo-hygrometric measurements with the development of biofouling appears difficult. However, the specific bio-construction situation was clearly influenced by the very environment in which it occurred, as reported in Appendix A.

Regarding the trend of salinity, its evolution tends to follow that of temperature, since higher evaporation rates concentrate the solution and lower rates reduce concentration. Furthermore, it can be observed how salinity variations were very different compared to those in the open sea outside of the bay in the area (data obtained from [27]). Kotor's bay exhibited lower salinity and greater oscillations due to the variable dilution with freshwater from rivers and other tributary sources, as described in [28] and again reported in Appendix A. Therefore, this suggests that the effect of this level of salinity on composite degradation is comparable to that obtained by their immersion in brackish water, which has been studied, e.g., in [29,30].

Direct hardness measurements were performed on both surfaces of the specimen to compare the fillers used and highlight any differences. For this purpose, the Shore D hardness was measured according to the ASTM D2240-21 standard [31] in five points per side of the sample (front and rear). Hardness was adopted as a replacement for tensile or flexural tests, since the extensive presence of bio-constructions over the six months would possibly result in wide variability in performance. Also, experimental data from samples of different configurations would be limited and possibly not very significant.

At this stage, it was possible to evaluate the weight variation as a result of loss of material and species growth, according to the following equation:

$$\Delta w = \left(\frac{w_a - w_b}{w_b} \right) \times 100 \quad (1)$$

where w_b indicates the initial weight of the specimen at fabrication and w_a represents its weight after a period of conditioning. Similarly, the same weighing procedure was initially used to evaluate the weight gain of the specimens after the immersion period and following the mechanical removal of protruding structures due to biofouling.

Regarding the evaluation of the state of degradation of the material, macro-photographic observations were carried out with the acquisition of high-resolution images before and after the storage period and observations under an EduBlue Stereo Microscope (Euromex, Arnhem, The Netherlands) with 5× or 10× magnification.

3. Results and Discussion

3.1. Preliminary Analysis on Posidonia oceanica Fibers

Before inserting the PO fibers into the resin, they were observed using SEM to evaluate their structure and possible compatibility with the epoxy resin, as limited information on this interaction is available so far. Preliminary studies were carried out, e.g., in [32], but in a general sense, not in a marine environment. In the images, it is possible to observe the surface morphology of the *Posidonia oceanica*, showing epidermal cells with elongated and thread-like shapes arranged in parallel. Additionally, a fair amount of porosity emerges, allowing the fibers to be incorporated into the epoxy resin (Figure 2).

In particular, in Figure 2a, a set of fibers extracted from the leaves of *Posidonia oceanica* can be observed; this shows the typical tape (flattened section) geometry, as recognized also in [33]. The fibers tend to be curved and difficult to arrange in a straight section because of their tendency to agglomerate. This is also advantageous for colonization, e.g., by diatoms, but it would pose further biofouling issues [34,35]. In Figure 2b, the cuticular microstructure of their epidermis is more clearly visible, and the outer fibrils, which tend to separate in bundles rather than as singularly. Bundle fibrillation, which has been revealed also in short stretches of other grass-extracted fibers, such as alfa fibers from esparto, provides mechanical interlocking, which is likely to improve the interface strength [36]. In general terms, the epidermis is able to offer rigidity and structural support to the leaf, helping it to withstand wave motion. It is also possible to observe sections of breakage due to fraying

of the leaf. In Figure 2c, the inset clarifies that the fibrillar fracture, though brittle, usually comes with some degree of separation, with very visible seams. White spots indicate the presence of bacteria and/or diatoms organized in biofilms on the fiber surface, including at the moment the fibers were collected [37,38]. In Figure 2d, a clear kink band is observable, with a cell thickening, which likely influenced the local strength of the fiber [39], and whose extent was possibly increased during the separation from the egagropili [40].

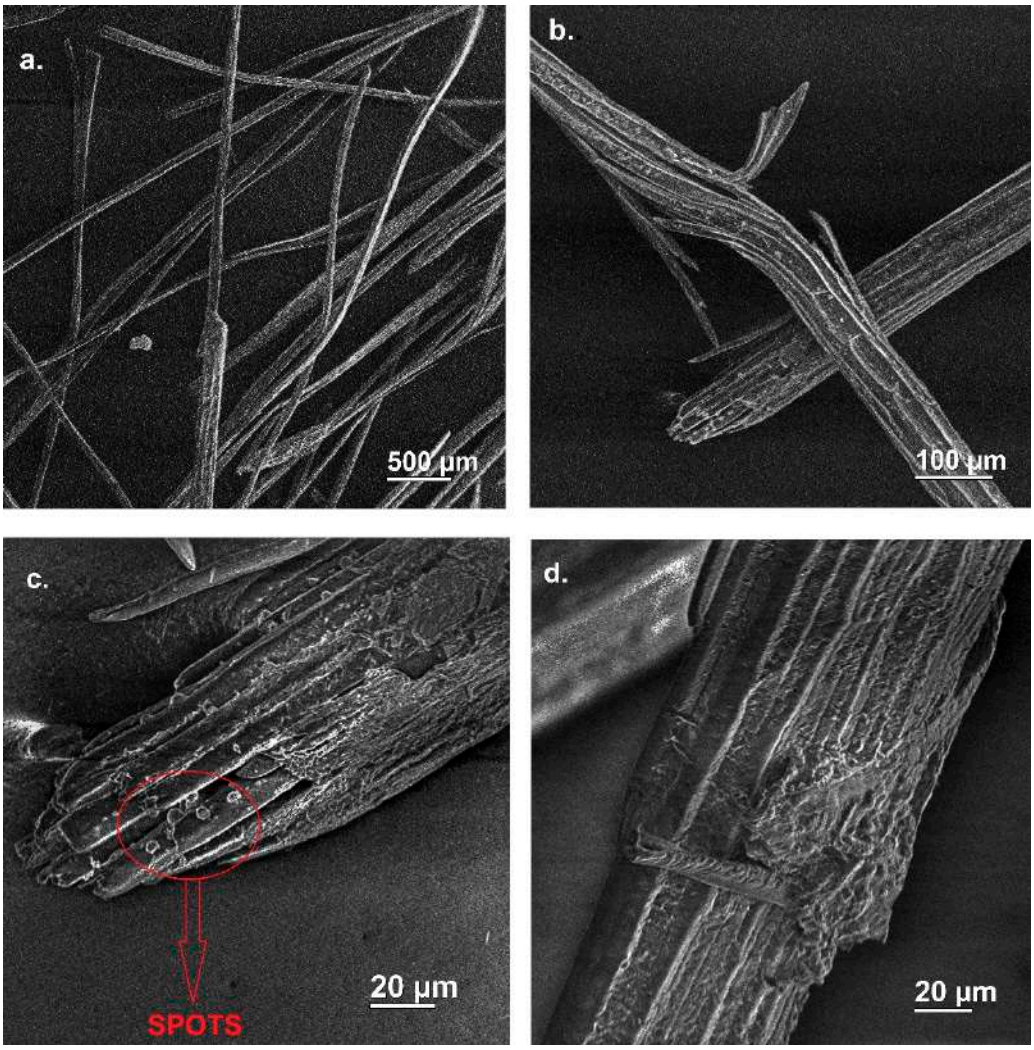


Figure 2. Observations of the surface morphology fiber of *Posidonia oceanica* by Scanning Electron Microscopy (SEM) at different magnifications. (**a**) PO fibers as extracted; (**b**) fibrillation behavior; (**c**) fibril seams; (**d**) kink bands.

3.2. Biofouling Analysis of Waste-Based Structures in Kotor's Bay

3.2.1. General Considerations

Marine biofouling refers to the accumulation of organisms on submerged surfaces, whether of artificial origin or natural substrates. The use of materials in the sea environment normally involves the gradual formation of biofouling organisms, as discussed in the introduction. In practice, a heterogeneous community of coexisting and interacting living organisms, or biocenosis, is formed and gradually develops, even in a very short time. This biological structure varies considerably in response to multiple environmental variables, such as temperature, salinity, water absorption, and interaction with other species. In particular, the specificity of the fouling community that develops on an exposed structure in the marine environment is influenced by the availability of native species at the dive site,

as well as by their effectiveness in establishing and thriving on the surface. Studies focused on the presence of golden coral in Kotor's Bay provide information on the specific biocenotic composition [41]. In particular, the physicochemical characteristics of the substrate exert an initial selective pressure that determines the composition of pioneer communities [42].

The complex surface of the composites is likely to be a possible host for bioconstructions, that is, the growth of structures by living organisms that rise from the seabed, physically and ecologically altering the environments [43]. Following the accumulation of successive generations of organisms, bioconstructions increase in volume and thickness, but at the same time, they can be gradually destroyed in the presence of demolishing organisms. Bioconstructors tend to form rigid structures with skeletons or shells by depositing calcium carbonate [44]. This is possible because the organisms absorb calcium bicarbonate ($Ca(HCO_3)_2$), which is readily available and dissolved in marine, river, and lake water, and synthesized it into calcium carbonate ($CaCO_3$) [45].

This evaluation of biofouling in the specific conditions of Kotor's Bay aims to investigate the resistance to sea exposure of a marine structure produced with local refuse as filler and expected to operate in the same area. It is worth noting that, within a circular economy approach, secondary raw materials should preferably not be transported, as this would generate additional environmental impact [46].

Specifically, the morphological and biological characterization of conditioned composites was conducted at two levels of biofouling analysis:

(i) Surface colonization, by mechanically removing and analyzing the organisms attached to the outer surface of the specimens;

(ii) Internal colonization, by examining the micro-pores of the composite materials to identify the pioneer colonies that had first penetrated and developed within the structure.

3.2.2. External Colonization Analysis

The samples were removed from the water in November 2024. Initial fouling analysis was conducted at the Institute of Marine Biology: the typical appearance of a sample after being exposed to the most aggressive conditions is depicted in Figure 3. The mass of the fouling community was determined using an analytical balance (VWR model LP-6202i, precision 0.01 g) by measuring the plate weight before and after fouling removal. Subsequently, the fouling material was detached from each plate, preserved in 70% ethanol, and taxonomically identified to the lowest possible level; when not possible, identification was limited to the genus. Nomenclature was aligned with the World Register of Marine Species (WoRMS).

Figure 3. Composite specimen after seawater exposure at a 2 m depth: the drilled hole for insertion into the carousel is shown on the left.

Analysis of the set of samples that were permanently submerged (at 0.5 m and 2 m depth) was carried out to assess the effect of prolonged use of these composites in this marine context, effectively serving as an accelerated aging procedure. This showed that fouling communities developed on all samples, but to varying extents, which is believed to have been primarily influenced by the material from which each plate was made. The weights of the colonies are reported in Figure 4. In the set of samples placed in the wave splash zone, no development of fouling communities occurred, as unfavorable conditions prevailed in that zone due to prolonged dry periods and high temperatures (Figure 5). The highest fouling mass was observed on plate S2 (54.62 g) (Figure 6a), while the lowest fouling mass was measured on plate BA2 (1.63 g) (Figure 6b).

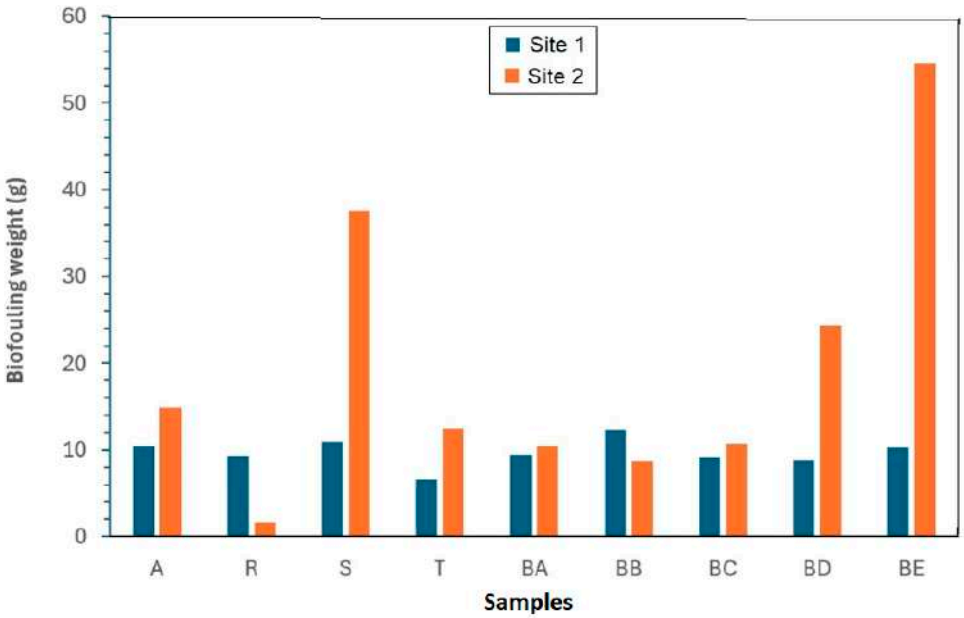


Figure 4. Weights of fouling communities developed on the sets of samples (expressed in grams, g) (at Site 3, the weight of fouling communities was negligible).

Figure 5. Test samples after six months of exposure: (**a**) samples retrieved from 0.5 m depth (Site 2), showing fouling communities; (**b**) samples from the splash zone (Site 3), with no fouling development due to desiccation and high temperature.

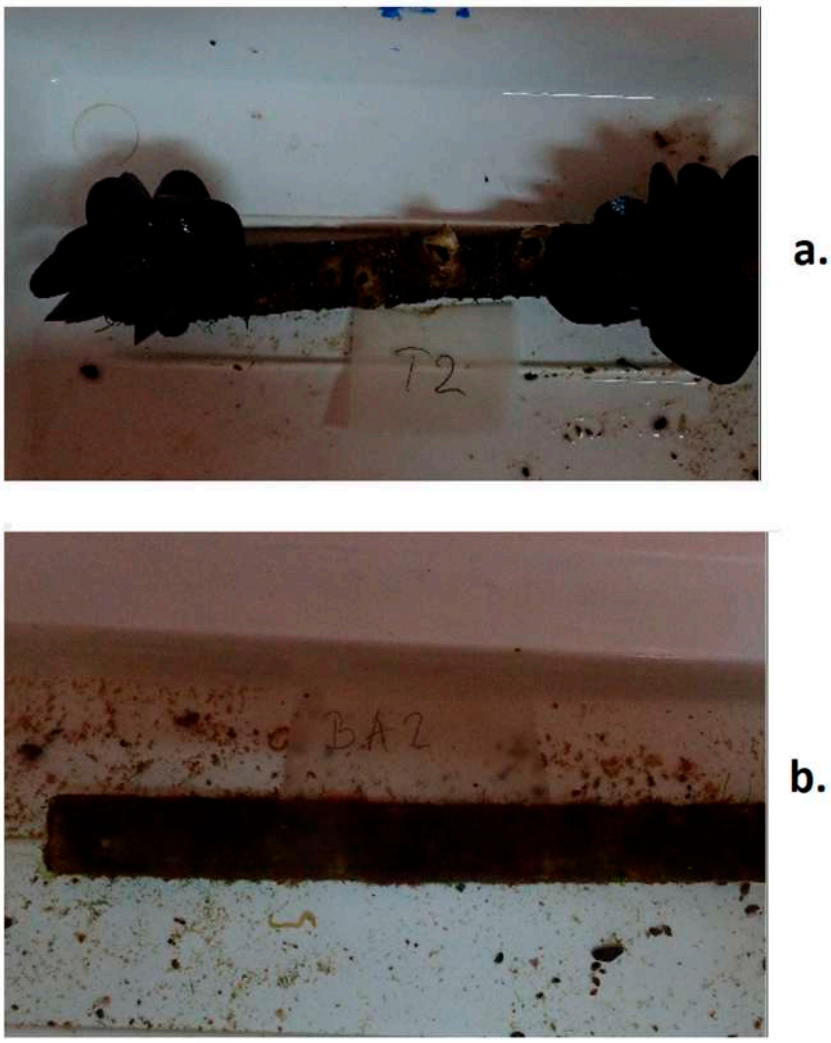


Figure 6. Test samples after seawater exposure: (**a**) plate S2 with a large cluster of *Mytilus galloprovincialis*; (**b**) plate BA2, showing the lowest fouling mass recorded.

Regarding the qualitative composition of the biofouling communities, a total of 13 species were identified, which indicates low diversity (as shown in Table 4. However, in terms of population size, the species *Mytilus galloprovincialis* and *Balanus perforatus* stood out, dominating and constituting the major portion of the fouling biomass. *Balanus perforatus* is largely present around the coasts of Eastern Adriatic and has often been related to be an environmental indicator of marine health, as reported e.g., in [47]. On sample S2, a large cluster of *Mytilus galloprovincialis* was present, so the fouling mass was measured both with and without this cluster.

Table 4. Taxonomic groups and identified species observed on samples after seawater exposure.

Taxonomic Groups	Representative Species Observed
Algae	*Sphacelaria* sp.
	Polysiphonia sp.
	Cladophora prolifera
	Enteromorpha sp.
Hydrozoans	*Eudendrium* sp.

Table 4. *Cont.*

Taxonomic Groups	Representative Species Observed
Worms (polychaetes)	*Spirorbis spirorbis*
	Janua heterostropha
	Anelida 1
	Anelida 2
Molluscs	*Mytilus galloprovincialis*
Arthropods	*Perforatus perforatus*
	Amphibalanus eburneus
Bryozoans	*Cryptosula pallasiana*

3.3. Analysis After Removal of Biofouling

It is worth noting that after mechanical removal of the biofouling structures, all the samples were still sufficiently sound and their geometry was mostly recognizable. This appears promising for the long-term durability of the composites.

Post-removal, three types of analysis were performed:

1. Weight measurement with respect to their initial (pre-immersion) weights to accurately assess whether the original material had been preserved;
2. Shore D hardness tests to verify by a simple and reasonably accurate method if some strength had been retained, considering that other tests (e.g., flexural testing) would lead to unreliable results for the point-to-point variability of the sample properties;
3. Macrophotographs and optical microscopy analysis to identify the surface differences sample by sample;
4. Each sample was weighed before immersion (*Starting Weight*) and was weighed again after retrieval (*Ending Weight*). The biofouling material was then removed, and the percentage of weight gain was calculated. The obtained results (Figure 7) indicate a clear influence of material composition and exposure conditions on the absorption behavior. In general, specimens immersed at greater depths exhibited higher water uptake and biofouling accumulation compared to those positioned in the splashing zone, where prolonged dry periods and high solar radiation limited the moisture content and reduced the fouling effect to almost nothing. Among all samples, the highest weight increases were recorded for samples from Site 1, such as S1 (7.70%), T1 (7.67%), T2 (7.62%), A2 (7.42%), and BE1 (6.59%), suggesting that the siting of these materials made them more susceptible to water penetration and subsequent biological colonization. Conversely, samples from Site 3, such as T3 (0.09%), S3 (0.32%), BE3 (0.49%), and U3 (−0.04%), showed negligible variation, indicating higher resistance to water uptake and reduced fouling settlement. These results confirm that the water absorption capacity strongly depends on the polymer matrix type and the inclusion of natural fillers, which may alter the porosity and permeability of the composites. Furthermore, the synergy between the solidity of the structure and biofouling development highlights the relevance of surface properties in marine applications, where prolonged exposure can significantly affect both dimensional stability and long-term mechanical performance.
5. Physical characterization, carried out by comparing the Shore D hardness of the samples and their weight variation (Δw) after a conditioning period, as from data reported in Table 5, was essential to observe the behavior of the composite. This allows ensuring its stability and durability and to understand how these materials react and degrade in response to environmental stimuli. From the calculation of the

weight variation before and after of the samples, at all the sites, despite the loss of material and following the removal of bioconstructions, some increases in weight still emerged. Only at Site 3, where the samples had a lower if not quasi-absent degree of colonization by marine species, a decrease in the weight of the samples was evident. In measuring the material's hardness, no evident differences between the samples were recorded. In some cases, namely Samples S at Site 1 and Site 2, and sample BE at Site 2, degradation did not allow for manual measurements to be taken on the front surface. There was a slight difference between the front surfaces, corresponding to the most degraded surfaces, and the rear ones, with a direct decrease in the hardness compared to the front. Samples S included 10% Posidonia, and their higher level of degradation was deemed to be due to the high salinity affecting the resin-confined lignocellulosic fibers when accessible to seawater [48]. It is also possible that for series T, with 15% Posidonia fibers, a higher level of adhesion with the matrix was obtained, which reduced the degradation effect.

6. After six months of composite conditioning and the mechanical removal of as many protruding bioconstruction structures as possible, one can observe from the comparison between the epoxy resin (samples A) and the eco-epoxy resin (samples BA) at the three sites that degradation occurred mainly at Site 2. In particular, sample A2 showed a high level of degradation, with erosion of the material and growth of *Balanus* sp. (barnacles), gastropods, mussels, *Lithophyllum incrustans* (coralline algae; a typical coralligenous outcrop of this marine area [49]), and algae. It is also clearly evident that the growth rate on the eco-epoxy resin was considerably lower, a difference that might depend on variations in porosities (Figure 8).

Table 5. Shore D hardness and weight variation (Δw) of the samples before and after immersion at Sites 1, 2, and 3 (average and standard deviation).

	Site 1 (2 m Depth)			Site 2 (0.5 m Depth)			Site 3 (Splashing Zone)		
Series	**Hardness (Front)**	**Hardness (Rear)**	**Δw (%)**	**Hardness (Front)**	**Hardness (Rear)**	**Δw (%)**	**Hardness (Front)**	**Hardness (Rear)**	**Δw (%)**
A	78.2 ± 2.4	74.2 ± 3.8	3.09	73.4 ± 4.4	72.0 ± 4.9	5.44	80.0 ± 0.7	77.6 ± 3.4	0.93
R	76.0 ± 2.0	75.2 ± 5.8	0.74	77.2 ± 2.6	76.2 ± 4.0	1.77	74.2 ± 3.7	73.0 ± 2.1	−0.08
S	- *	75.2 ± 6.1	0.58	- *	76.6 ± 3.9	3.04	76.0 ± 4.3	79.2 ± 3.1	−0.49
T	76.0 ± 3.2	74.4 ± 4.5	1.12	75.0 ± 3.2	77.2 ± 1.6	4.28	77.4 ± 2.1	77.6 ± 1.8	−0.70
BA	76.2 ± 0.8	76.2 ± 1.6	1.11	76.6 ± 1.9	75.4 ± 0.9	1.26	77.6 ± 2.4	77.8 ± 2.5	0.64
BB	78.8 ± 3.8	79.0 ± 1.0	1.23	76.4 ± 4.3	76.2 ± 2.2	3.13	74.0 ± 2.5	78.8 ± 3.3	0.48
BC	77.2 ± 2.7	77.8 ± 2.4	0.60	76.8 ± 2.5	74.8 ± 3.1	2.51	77.0 ± 2.9	75.8 ± 3.8	0.54
BD	83.0 ± 0.7	74.6 ± 4.0	0.19	80.4 ± 1.5	77.8 ± 1.3	2.10	82.6 ± 1.1	77.6 ± 1.1	0.34
BE	75.2 ± 1.8	75.0 ± 2.4	0.11	- *	75.6 ± 1.1	1.74	73.2 ± 2.5	75.4 ± 3.8	−0.49

* Sample degradation did not allow for manual measurements to be taken.

In the samples prepared with the incorporation of *Posidonia oceanica* in eco-epoxy resin at 5%, 10%, and 15%, a definite increase in marine colonization growth was observed at all exposure sites as the amount of incorporated waste increased, as shown in Figure 9. This can be considered as a natural colonization process since, in nature, *Posidonia oceanica*, with its underwater meadows, provides one of the most important and biodiverse habitats in the Mediterranean, hosting a wide range of organisms that grow and find refuge there, especially in the summer. In particular, the surface of the leaves and rhizomes of this species are frequently colonized by a characteristic epiphytic community, including foraminifera, encrusting coralline algae, diatoms, cyanobacteria, and bacteria [50–52].

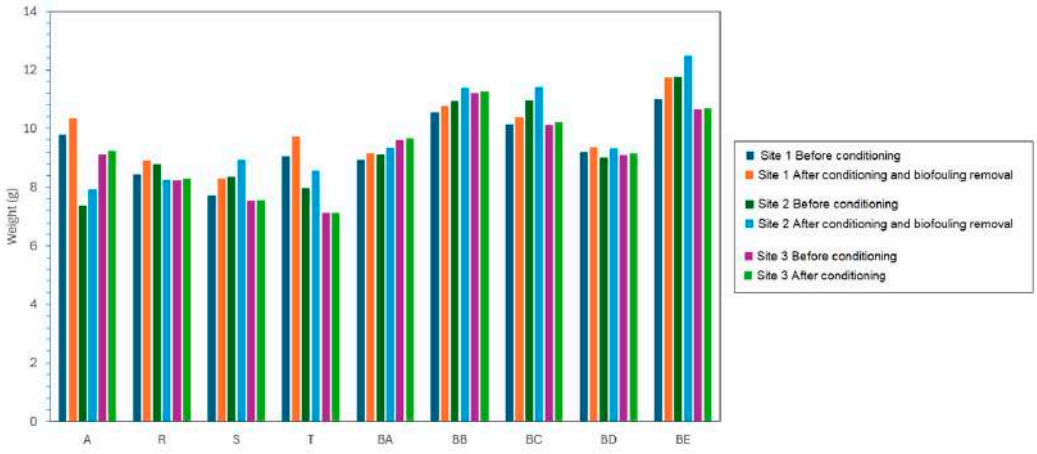


Figure 7. Initial and final weights of the specimens and corresponding weight gain (%) after six months of marine exposure and following the removal of the biofouling structures.

Figure 8. Comparison between epoxy resin (samples A) and eco-epoxy resin (samples BA) after six months at the three different sites: front surface (**left**), and rear surface (**right**).

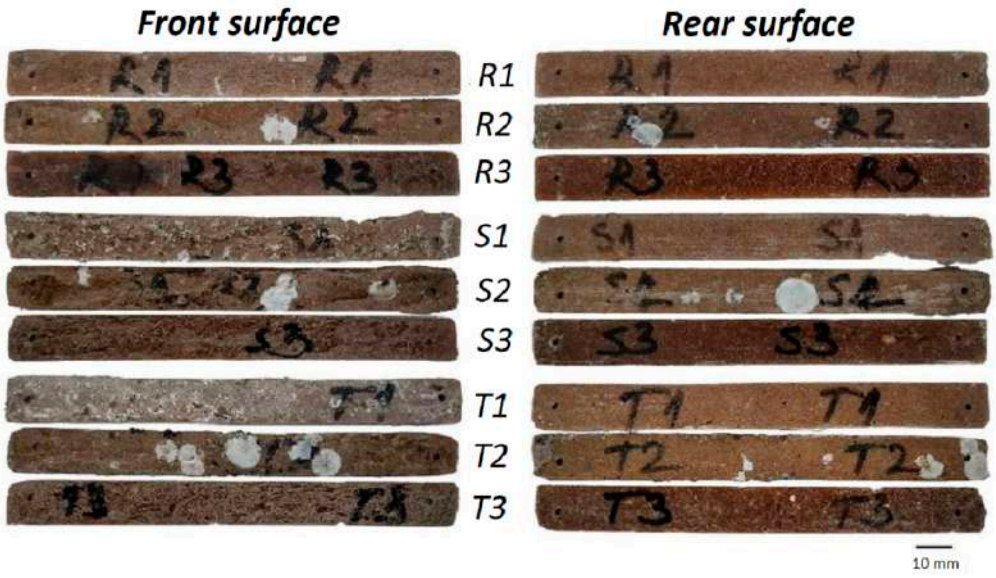


Figure 9. Comparison of epoxy resins with 5%, 10%, and 15% PO filler, corresponding to samples R, S, and T, respectively, after six months at the three different sites: front surfaces (**left**) and rear surfaces (**right**) of the samples are reported.

From the comparison of the samples filled with marine secondary raw materials, greater stability emerged for those containing oysters and clams, while extensive colo-

nization and deformations appeared in those filled with mussels. In contrast, the material loaded with *Posidonia oceanica* showed greater degradation and wearing, as well extensive colonization at Site 2. Details are provided in Figure 10.

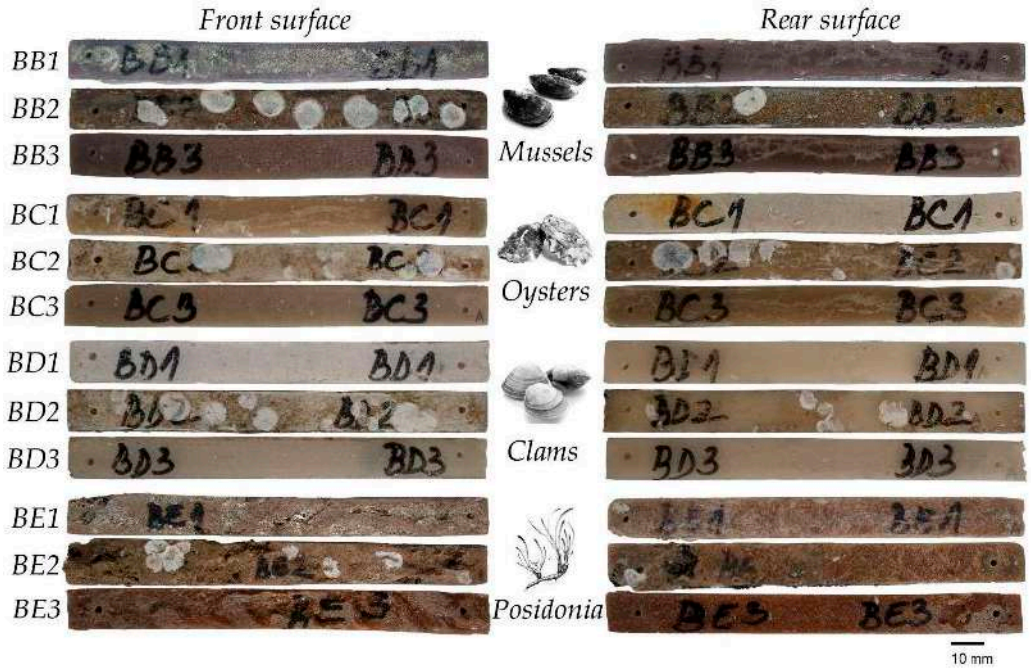


Figure 10. Comparison of epoxy resin with 15% filler (mussels, oysters, clams, or *Posidonia oceanica*) in samples BB, BC, BD, and BE, respectively, after six months at three different sites: front surface (**left**) and rear surface (**right**).

An overall observation is that numerous marine growths were observed at Sites 1 and 2, as well as degrading phenomena deriving from marine conditions (Figure 11). In particular, the development of colonies (probably *Bryozoans*), gastropods, mussels, and green and red algae were observed. It is important to remember that the biofouling process begins with the initial adhesion of organic and inorganic particles to surfaces, followed by the colonization, growth, and accumulation of primary microorganisms (e.g., bacteria, diatoms). Subsequently, additional microorganisms and macro-organisms led to the progressive stratification and maturation of the biofilm [53]. In general, the colonization of new surfaces is initially dominated by *Serpulids*, which have high growth rates. Subsequently, the community undergoes a transition in which *Serpulids* are completely or almost completely supplanted by mussels and partially by bryozoans, organisms that co-colonize the substrate in the initial phases, and whose presence is a symptom of insufficient protection from biofouling [54].

In the case considered, *Balanus* sp. (barnacles) and algae growth were found only in the samples at Site 2. No growth of colonies or isolated individuals was observed at Site 3. Only degradation was observed, linked to the erosion of the material (probably due to climatic variations during the cycle), deformations, and superficial deposits of salts. As discussed above, Figure 11 shows an overall view of the species grown on the specimens in relation to the storage site to provide greater clarity on what was optically found. It is fair to say that the degradation of epoxy resin composites in a marine environment is largely influenced by the pH value, which is relatively constant in an open sea and even more so in an oceanic environment, yet quite variable in a harbor context, and especially in low-salinity water. In practice, it was found that under simulated water conditions, the degradation of epoxy resin increased in highly alkaline conditions, reaching important percentages (such as over 20% tensile strength loss at pH = 12.6) [55]. It was considered important to evaluate

this bio-fouling study in the specific context in which, following a "zero km" approach and respecting the circular economy, local waste-loaded epoxy composites would possibly be used.

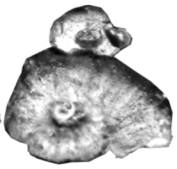
SAMPLES	 Balanus sp.	 Developing colonies	 Gastropods	 Mussels	 Algae
Site 1					
A1	-	-	X	-	-
R1	-	X	X	-	-
S1	-	X	X	X	-
T1	-	X	X	X	X
BA1	-	-	X	-	-
BB1	-	X	X	X	-
BC1	-	X	X	-	-
BD1	-	X	-	-	-
BE1	-	X	X	X	-
Site 2					
A2	X	-	X	X	X
R2	X	X	X	X	-
S2	X	X	X	X	X
T2	X	X	X	X	X
BA2	X	-	-	-	X
BB2	X	-	-	X	X
BC2	X	X	-	X	X
BD2	X	X	-	X	X
BE2	X	X	X	X	X
Site 3					
A3	-	-	-	-	-
R3	-	-	-	-	-
S3	-	-	-	-	-
T3	-	-	-	-	-
BA3	-	-	-	-	-
BB3	-	X	-	-	-
BC3	-	-	-	-	-
BD3	-	-	-	-	-
BE3	-	-	-	-	-

Figure 11. Details of marine growth (indicated by X) on the single samples divided by site (after mechanical removal of biofouling).

From the images obtained using an optical microscope, barnacle development on the surface of the samples, particularly at Site 2, was observed, which can take place in an isolated manner or by forming aggregate colonies. In particular, occurrences on the Posidonia Oceanica-filled samples are reported in Figure 12, while those developed in mollusk powder-filled samples are depicted in Figure 13. Among the most relevant parameters for barnacle adhesion are the characteristics of the adhering surface, namely chemical properties (wettability, free energy, electrical charge) and mechanical properties (e.g., stiffness and roughness). Barnacles are among the most encrusting marine organisms on hard substrates. During their life cycle, adhesion phenomena are fundamental, particularly in the transition from the larval stage to the sessile adult stage. The entire adult stage,

crucial for reproduction, also depends strictly on adhesion to a wide variety of substrates. It has been observed elsewhere that epoxy provides surface properties suitable for barnacle adhesion, as opposed to other plastics, such as silicone [56]. The chemical mechanism underlying barnacle adhesion is complex and involves several classes of substances, such as lipids, antimicrobial peptides, and binding proteins. Some barnacles, such as *Balanus* sp. in the presented case, produce a calcareous basal plate in the adult stage; as the basal plate expands radially and the barnacle undergoes periodic molts, the adhesive must be continuously produced, making adhesion a persistent challenge in their life cycle. In general, barnacle adhesion can be considered an adhesive-based system. If the adhesive can easily wet the surface, increased roughness usually leads to an increase in the effective contact area. This results in a mechanical interlocking between the cured adhesive and the rough solid substrate, which further increases the bond strength compared to a smooth substrate [57].

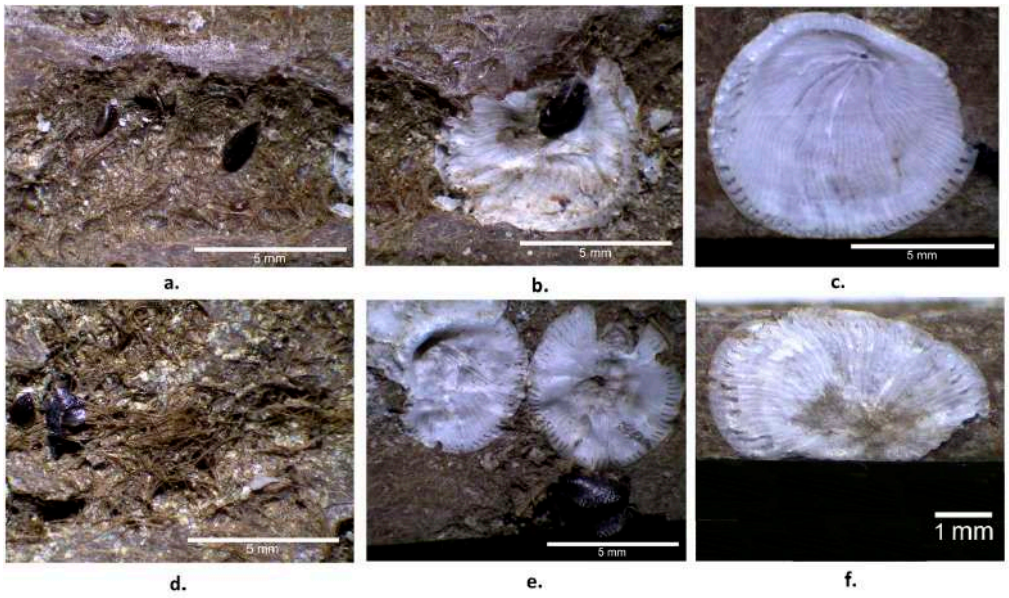


Figure 12. Optical microscope view of the samples showing the growth of *Balanus* sp. (barnacles) in isolated form and in colonies, adhering to the surface with radial growths on epoxy samples containing PO fibers. (**a**–**c**) S2 sample; (**d**–**f**) T2 sample.

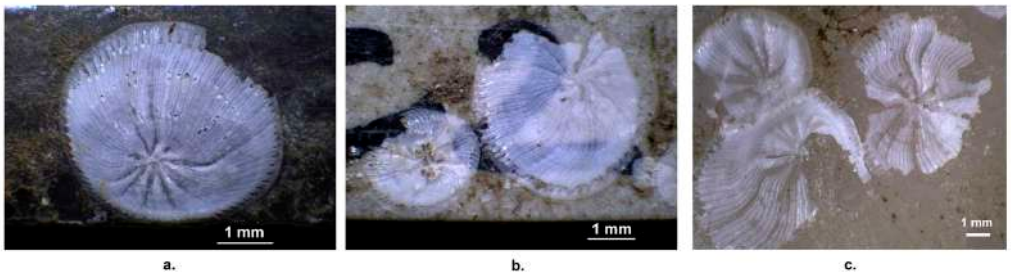


Figure 13. Optical microscope view of the samples showing the growth of *Balanus* sp. (barnacles) in isolated form and in colonies, adhering to the surface with radial growths on eco-epoxy samples containing mollusk powder. (**a**) BB2 sample (with mussel powder); (**b**) BC2 (with oyster powder); (**c**) BD2 sample (with clam powder).

Regarding other encrustations present on the samples after removal of the bulk of the biofouling, the development of extensive microscopic colonies from Site 1 and Site 2 was revealed, whose identification was not possible due to their death following removal from water. From the residual morphology observed in Figure 14, these were probably *Bryozoans*, organisms that settle almost synchronously with *Serpulids*, and with a typical

mid- to late-summer peak. They often show an initial dominance in the first phases of colonization and are then replaced by mussels and bryozoans [58].

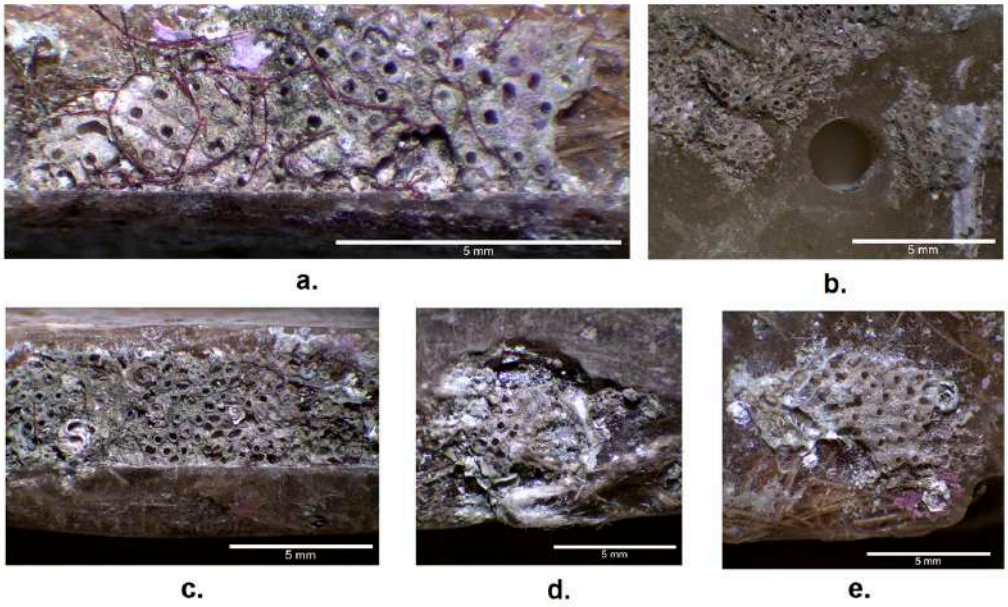


Figure 14. Optical microscope view of the samples showing the initial stages of development of aggregated microcolonies: (**a**) sample T1; (**b**) sample BC1; (**c**–**e**) sample BE1.

Another species that colonized the samples belongs to the large family of gastropods, which are among the largest groups in the marine benthos. In marine ecosystems, gastropods can play different roles depending on the species, such as filter feeders, phytophagous, predators, or parasites. Here, at this stage of immersion, micro-gastropods or micro-mollusks were observed, that is, gastropods with adult dimensions equal to or less than 5 mm, which could facilitate bio-erosion [59]. The latter is widely considered one of the principal limitations to the broad introduction of bio-based materials into composite marker whenever their use in water is involved [60]. It is interesting to note how in our samples, the presence of gastropods inhibited the growth of barnacles and algae by facilitating the removal of the biofilm layer of colonization, which promotes the development of microorganisms, or by occupying the entire available surface, thus hindering the presence of other species [61]. This occurs especially in relatively confined environments, such as estuarine or fjord contexts [62]. This phenomenon occurred particularly in samples with Posidonia fiber and mussel powder filling, that is, samples S1, T1, BB1, and BE1, in which the presence of other species was not found except in the initial stages of colonization, with the exception of mussels (Figure 15).

Mussels are among the most problematic and studied macrofouling organisms on substrates in marine environments. This genus of organisms is able to rapidly colonize large surfaces by forming dense aggregations. Compared to other invertebrates, such as serpulid worms and barnacles that cement their calcareous shells on substrates, mussels secrete byssus, a bundle of flexible protein filaments produced by a foot gland, which allows for the anchoring of the soft body of the mussel to the substrate [63]. The growth of mussels was particularly vivacious on the samples filled with Posidonia, probably due to the rougher substrate and denser fibrousness similar to Posidonia byssus, which allowed for their cohesion and development in isolated or aggregate forms (Figure 16).

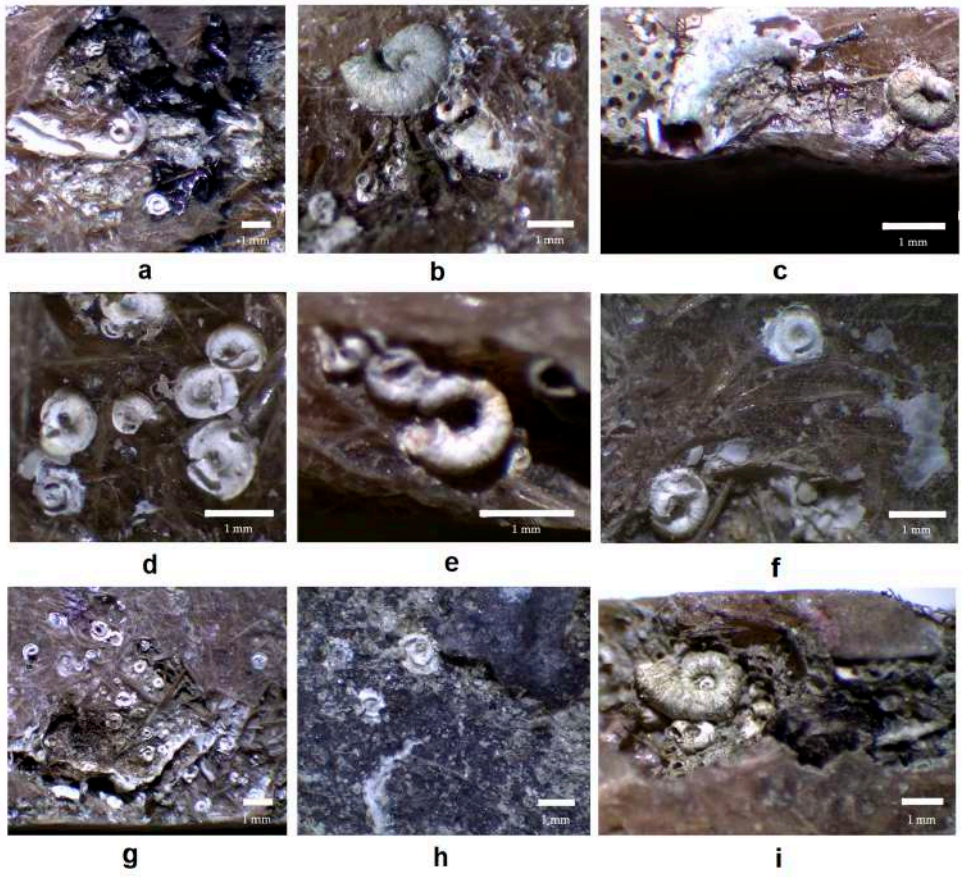


Figure 15. Optical microscope view of the samples showing the growth of microgastropods: (**a**–**e**) sample S1; (**f**,**g**) sample T1; (**h**) sample BB1; (**i**) sample BE1.

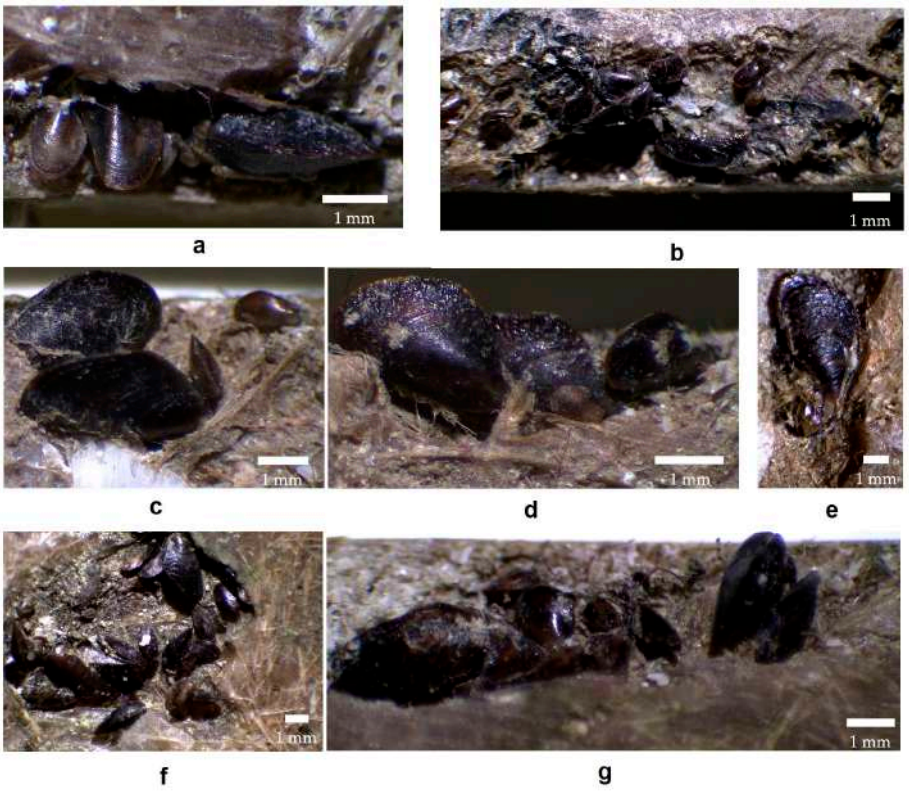


Figure 16. Optical microscope view of the samples showing the growth of *Mytilus galloprovincialis* (mussels): (**a**) sample S2; (**b**) sample S1; (**c**,**d**) sample T2; (**e**,**f**) sample BE2; (**g**) sample BE2.

Marine fouling algae, particularly those belonging to green algae (*Chlorophyta*), red algae (*Rhodophyta*), and coralline algae (a subgroup of red algae), are important agents of biofouling on submerged surfaces. Algae are among the first colonizers of submerged surfaces through motile microscopic spores for the formation of microbial biofilms (bacteria and diatoms). Their presence is crucial in the early stages of fouling succession and can influence the establishment of subsequent organisms [64–66]. From submerged samples, traces of branched green and red algae were found, while among the most microscopically present were coralline algae, especially near the edges of the samples, where they also favored the growth of mussels, as can be seen in Figure 17.

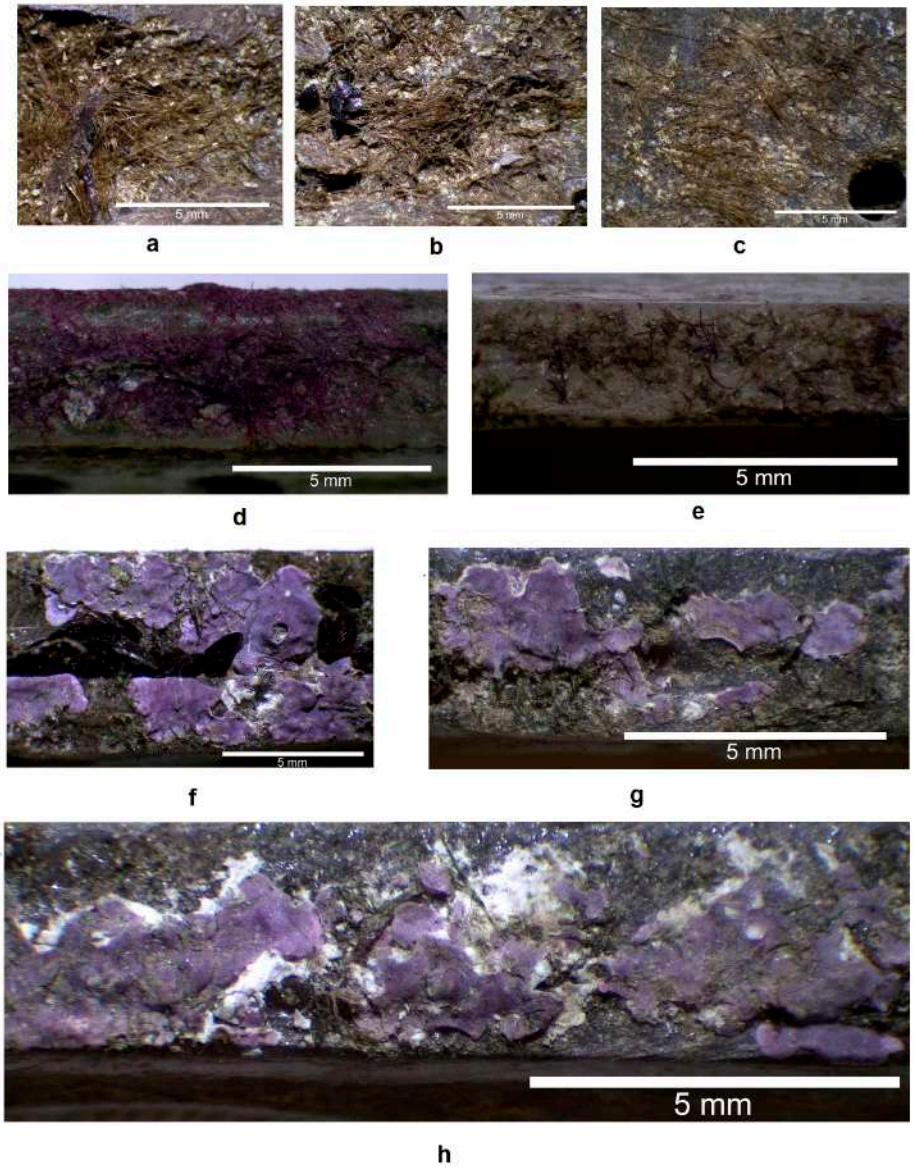


Figure 17. Optical microscope view of the samples showing the growth of algae. Green algae: (**a**) sample S2; (**b**) sample T2; (**c**) sample BB2. Red algae: (**d**) sample BA2; (**e**) sample BD2. Coralline algae: (**f**) sample BE2; (**g**) sample BB2; (**h**) BB2 sample.

The research revealed that the epoxy resin specimens loaded with fillers and placed at Site 3 did not show any particular problem with non-continuous contact with seawater. Specifically, very limited biofouling contamination and no significant degradation attributable to atmospheric phenomena were observed. These results suggest that the composites can be suitable for applications such as fishing boat seats. Furthermore, no sig-

nificant differences were found among the specimens at Site 3 resulting from the fillers used, including Posidonia fibers, whose application was suggested to be critical for the microscopical structure observed and proneness to biological wear and eventual fragmentation. However, on this timescale, these issues were still marginal.

With exposure at Site 1 and Site 2, high contamination by *Balanus* sp., in addition to the other species, as previously highlighted, was revealed. However, it is important to underline that, as anticipated in the Introduction, Site 1 and Site 2 represented extreme environmental conditions in which the specimens were tested.

4. Conclusions

Several observations were made from composites manufactured using bio-epoxy resins with various fillers (mussel, oyster, clam powder, and Posidonia fibers) up to 15 wt.%. The composites were tested in marine conditions using composite beams for practical and durable exposure at three different seawater sites and characterized over a six-month period. This occurred in the context of Kotor's Bay, Montenegro, where the same fillers were obtained as secondary raw materials: the bay water presents conditions of low salinity, not normally exceeding 20 PSU. The composites demonstrated acceptable resistance to degradation at Site 3 (on the pier), which is not uniformly wet, especially during day–night cycles. In this environment, little biofouling contamination and no significant degradation due to weathering were observed. Among the configurations examined, more serious issues were observed in the samples containing *Posidonia oceanica* fibers, indicating a more significant trend toward degradation than the other composites.

These results confirm the suitability of these composites for specific applications within a vessel, where they are intended to have only a discontinuous seawater wetting. In contrast, Site 1 (on the bottom of the sea in the harbor area, at around a 2 m depth) and Site 2 (completely immersed, at around a 0.5 m depth) represented extreme environmental conditions. In these positions, the severity of biofouling was confirmed, indicating that the application of some form of protection is required, despite the fact that seawater conditioning did not produce any significant reduction in the samples' hardness.

In view of the prospective applications of marine waste materials in these composites for applications within the same environment, i.e., eastern regions of Adriatic sea, the results may appear promising. However, intensifying the conditions would clearly result in larger amounts of biofouling and, in the case of introducing *Posidonia oceanica* fibers, also in significant wearing of the surfaces. However, it is important to note that the introduction of a larger amount of waste in the matrix and/or the modification of the polymer used, which are amongst the future objectives of the project, might lead to exacerbating the issues highlighted. These include not only the formation of biofouling, considering that epoxy is a preferred polymer surfaces for their adhesion, but also the possible consumption of the samples by bio-erosion.

Author Contributions: Conceptualization, D.N., C.S., A.P., and C.F.; methodology, R.G., S.P., and C.F.; validation, A.P., C.S., G.V., S.M., and A.F.; investigation, G.V., R.M., S.M., S.P., R.G., and A.F.; resources, D.N., R.M., A.P., C.S., and C.F.; writing—original draft preparation, G.V., S.M., and C.S.; writing—review and editing, G.V., S.M., C.S., and C.F.; supervision, D.N., A.P., C.S., and C.F.; project administration, D.N., C.S., and C.F.; funding acquisition, D.N. and C.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded by the Ministry of Foreign Affairs and International Cooperation of Italy and by the Ministry of Education, Science, Culture and Sports of Montenegro, as part of the bilateral Science and Technology Cooperation Program 2022–2024, under the project entitled 'SEA-COMP, Sea Waste from Adriatic to Enhance Marine Composites'.

Data Availability Statement: Data is available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ASTM	American Society for Testing and Materials
CDW	Construction and demolition waste
Δw	Weight variation
PO	*Posidonia oceanica*
PSU	Practical salinity unit
SEM	Scanning electron microscope
T_{atm}	Atmospheric temperature
T_{sea}	Sea surface temperature
w_a	Sample weight after sea conditioning
w_b	Sample weight at fabrication

Appendix A

During the period of monitoring, an average T_{atm} of 22.5 °C was recorded with a standard deviation of 3.88, T_{sea} of 23.95 ± 2.49, S_{sea} of 38.04 ± 0.61, and S_K of 19.78 ± 4.66; with maximum peaks of 37 °C for T_{atm}, 29.2 °C for T_{sea}, S_{sea} 34.86 PSU, and S_K 31.06 PSU; and minimums of T_{atm} 14.5 °C, T_{sea} 18.2 °C, S_{sea} 16.8 PSU, and S_K 8.56 PSU.

Clear evidence shows that oscillations between T_{sea} and T_{atm} are slower in the sea than in the atmosphere. It is also important to note that, until the beginning of September, the difference between atmospheric temperature and sea temperature might be positive or negative, while the latter is consistently higher from September on. This is because the variations are much slower and more gradual than in the atmosphere due to the large thermal capacity of water (Figure A1). Monthly averages and relevant standard deviations are shown in Figure A2. The oscillations between the maximum and minimum atmospheric temperatures are given in Figure A3. These figures may provide insights into the respective development of biofouling at different sites of conditioning. Salinity data from the open sea at the entrance of Kotor's bay, as reported in [27], are reported in Figure A4. Monthly average salinity levels and standard deviations are reported in Figure A5.

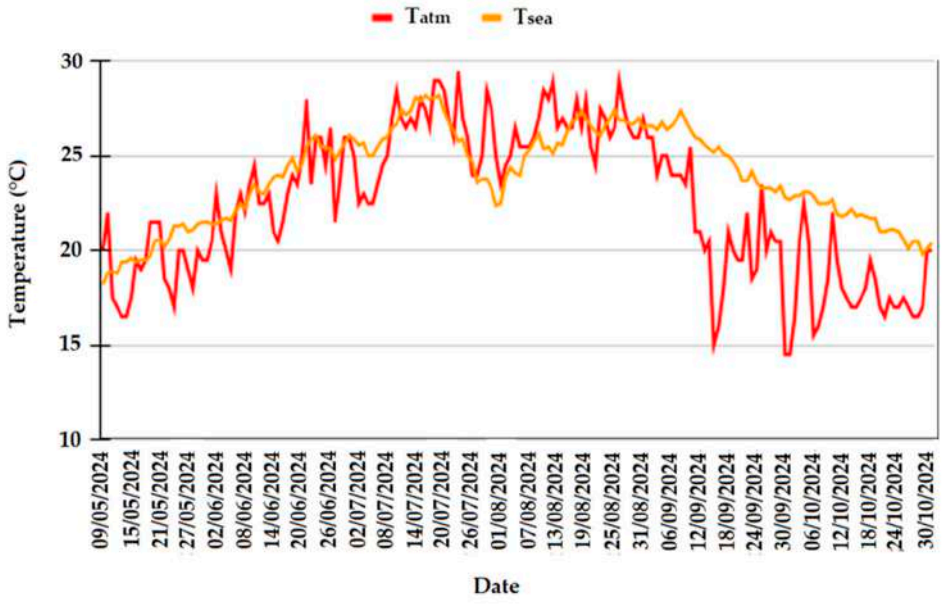


Figure A1. Comparison between daily atmospheric and seawater temperature (T_{atm} and T_{sea}).

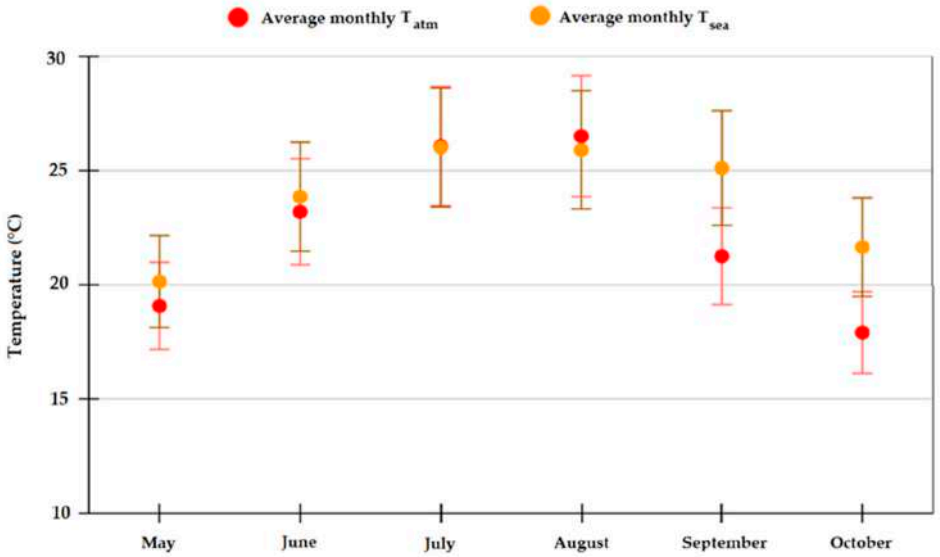


Figure A2. Comparison between monthly average atmospheric and seawater temperature.

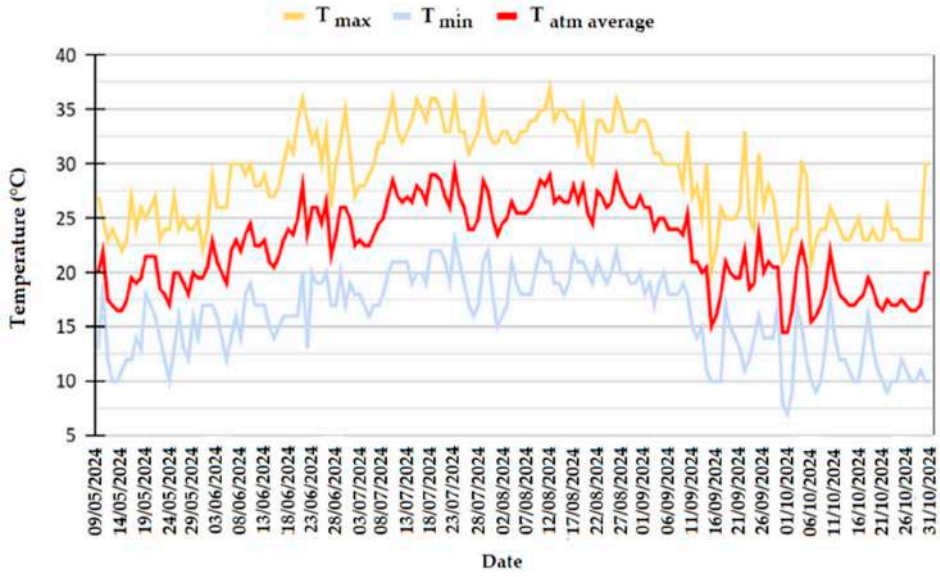


Figure A3. Atmospheric thermal oscillations.

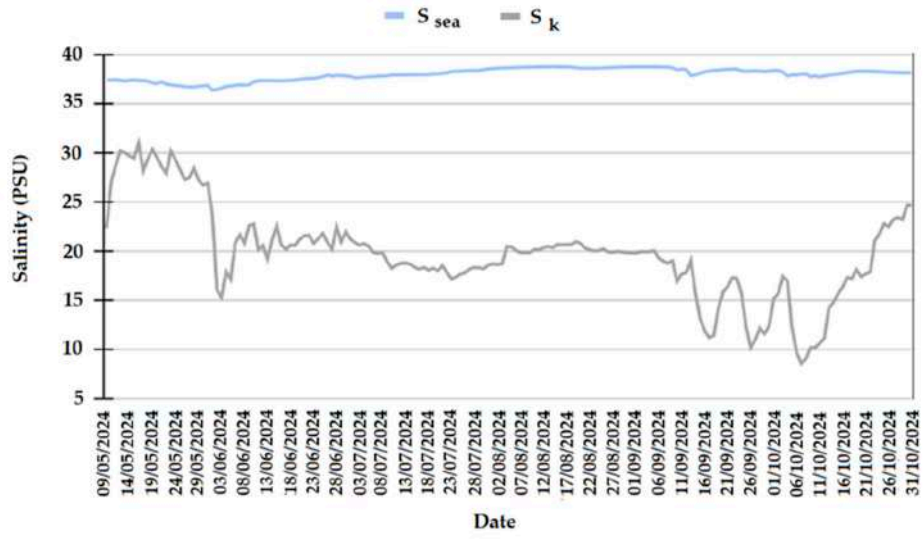


Figure A4. Daily variation in marine salinity (data from [27]) in the open sea and Kotor's Bay.

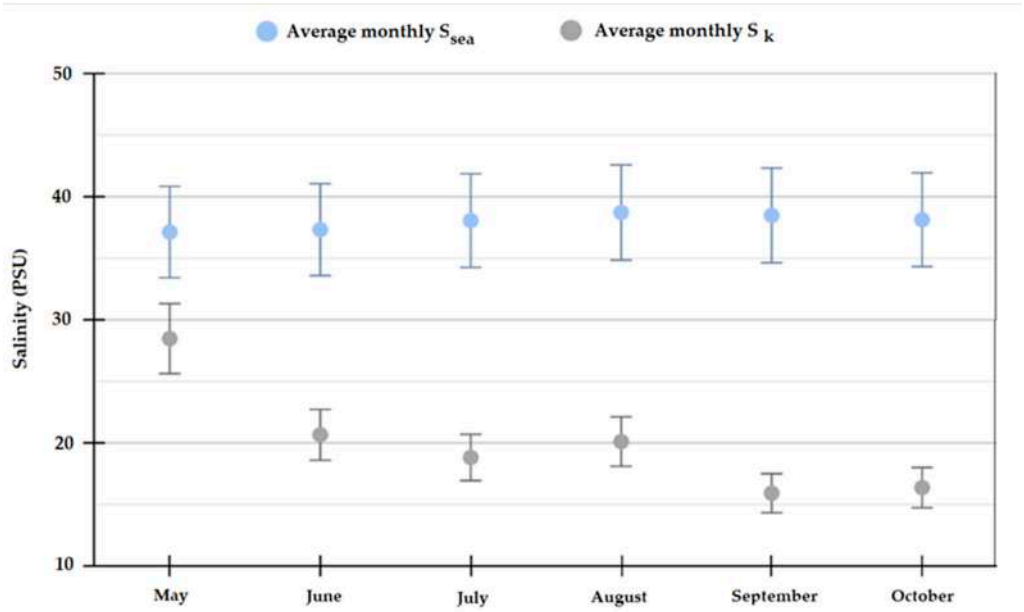


Figure A5. Monthly variation in marine salinity in the open sea and Kotor's Bay.

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