

## Article

# Response of Hard-Bottom Macro-Zoobenthos to the Transition of a Mediterranean Mariculture Fish Plant (Mar Grande of Taranto, Ionian Sea) into an Integrated Multi-Trophic Aquaculture (IMTA) System

Roberta Trani <sup>1</sup>, Cataldo Pierri <sup>1,\*</sup>, Antonella Schiavo <sup>1</sup>, Tamara Lazic <sup>1</sup>, Maria Mercurio <sup>1</sup>, Isabella Coccia <sup>2</sup>, Adriana Giangrande <sup>3</sup> and Caterina Longo <sup>1</sup>

<sup>1</sup> Department of Bioscience, Biotechnology and Environment, University of Bari Aldo Moro, Via Orabona 4, 70125 Bari, Italy; roberta.trani@uniba.it (R.T.); a.schiavo2@phd.uniba.it (A.S.); tamara.lazic@uniba.it (T.L.); maria.mercurio@uniba.it (M.M.); caterina.longo@uniba.it (C.L.)

<sup>2</sup> Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis, 86100 Campobasso, Italy; isabella.coccia@unimol.it

<sup>3</sup> Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Provinciale Lecce-Monteroni, 73100 Lecce, Italy; adriana.giangrande@unisalento.it

\* Correspondence: cataldo.pierri@uniba.it

**Abstract:** This study investigates the effects on hard-bottom macro-zoobenthic communities of converting a mariculture plant into an Integrated Multi-Trophic Aquaculture (IMTA) system. This study was conducted from 2018 to 2021 in the semi-enclosed Mar Grande basin of Taranto (northern Ionian Sea), on a facility located 600 m off the coastline, with a production capacity of 100 tons per year of seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*). The results from seasonal sampling performed in a treatment site, where bioremediators (filter-feeding invertebrates such as sponges, polychaetes, mussels, and macroalgae) were deployed, and a control site without bioremediators were compared. Before the IMTA installation, the hard substrates under the cages were sparsely inhabited, with significant sediment coverage. By 2021, the treatment site exhibited revitalized and more diverse macro-zoobenthic communities, with species richness increasing from 83 taxa in 2018 to 104 taxa, including notable growth in sponges, annelids, mollusks, and bryozoans. In contrast, the control site showed no substantial changes in biodiversity over the same period. Biodiversity indices, including Shannon and Margalef indices, improved significantly at the treatment site, particularly during summer months, highlighting a more resilient and balanced benthic environment. Taxonomic distinctness (delta+) and multi-variate analyses (PERMANOVA, PCO) confirmed significant spatial and temporal shifts in community structure at the treatment site, driven by IMTA implementation. SIMPER analysis identified key taxa contributing to these changes, which played a pivotal role in structuring the community. The emergence of filter feeders, predators, and omnivores at the treatment site suggests enhanced nutrient cycling and trophic complexity, while the decline in opportunistic species further supports improved environmental conditions. These findings demonstrate the potential of IMTA to promote recovery and sustainable mariculture practices, also offering a comprehensive understanding of its positive effects on hard-bottom benthic community dynamics.

**Keywords:** hard-bottom macro-zoobenthos; IMTA; biodiversity; innovative mariculture; zooremediation; environmental health; restoration



Academic Editor: Jean-Claude Dauvin

Received: 11 December 2024

Revised: 9 January 2025

Accepted: 12 January 2025

Published: 15 January 2025

**Citation:** Trani, R.; Pierri, C.; Schiavo, A.; Lazic, T.; Mercurio, M.; Coccia, I.; Giangrande, A.; Longo, C. Response of Hard-Bottom Macro-Zoobenthos to the Transition of a Mediterranean Mariculture Fish Plant (Mar Grande of Taranto, Ionian Sea) into an Integrated Multi-Trophic Aquaculture (IMTA) System. *J. Mar. Sci. Eng.* **2025**, *13*, 143. <https://doi.org/10.3390/jmse13010143>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Mariculture plays a crucial role in global protein supply, particularly as the demand for sustainable food sources continues to rise [1]. As the global population grows, mariculture offers a sustainable alternative to land-based systems, reducing pressure on terrestrial ecosystems while meeting the increasing demand for animal protein [2].

On the other hand, mariculture has garnered growing attention due to its potential impacts on the marine environment [3–5]. Indeed, the load of organic matter from mariculture can alter the structure and function of benthic communities, with long-term effects on ecosystem resilience and recovery [6]. The diversity of macro-zoobenthos in mariculture areas is influenced by complex interactions of both biotic and abiotic factors, such as nutrient availability, sediment characteristics, temperature variations, and anthropogenic activities like nutrient loading from aquaculture. These factors impact species composition and the health of these communities, as macro-zoobenthos play key roles in nutrient cycling, sediment modification, and overall ecosystem stability [7,8]. Changes in conditions, such as eutrophication or pollution, can cause shifts in abundance and biodiversity, which can lead to both positive and negative ecosystem effects depending on the stressors involved [9,10].

The shift towards more sustainable aquaculture practices has led to increased interest in Integrated Multi-Trophic Aquaculture (IMTA) systems, which aim to mitigate the environmental impacts of traditional mariculture by fostering a balanced ecosystem approach [11]. IMTA systems integrate species from different trophic levels, combining two key components, fed species, such as fish or shrimp, and extractive species, such as edible and non-edible bioremediators (including sponges, polychaetes, tunicates, holothurians shellfish and algae), to optimize nutrient recycling and reduce waste, offering a potential solution to the negative ecological effects commonly associated with conventional farming system [11].

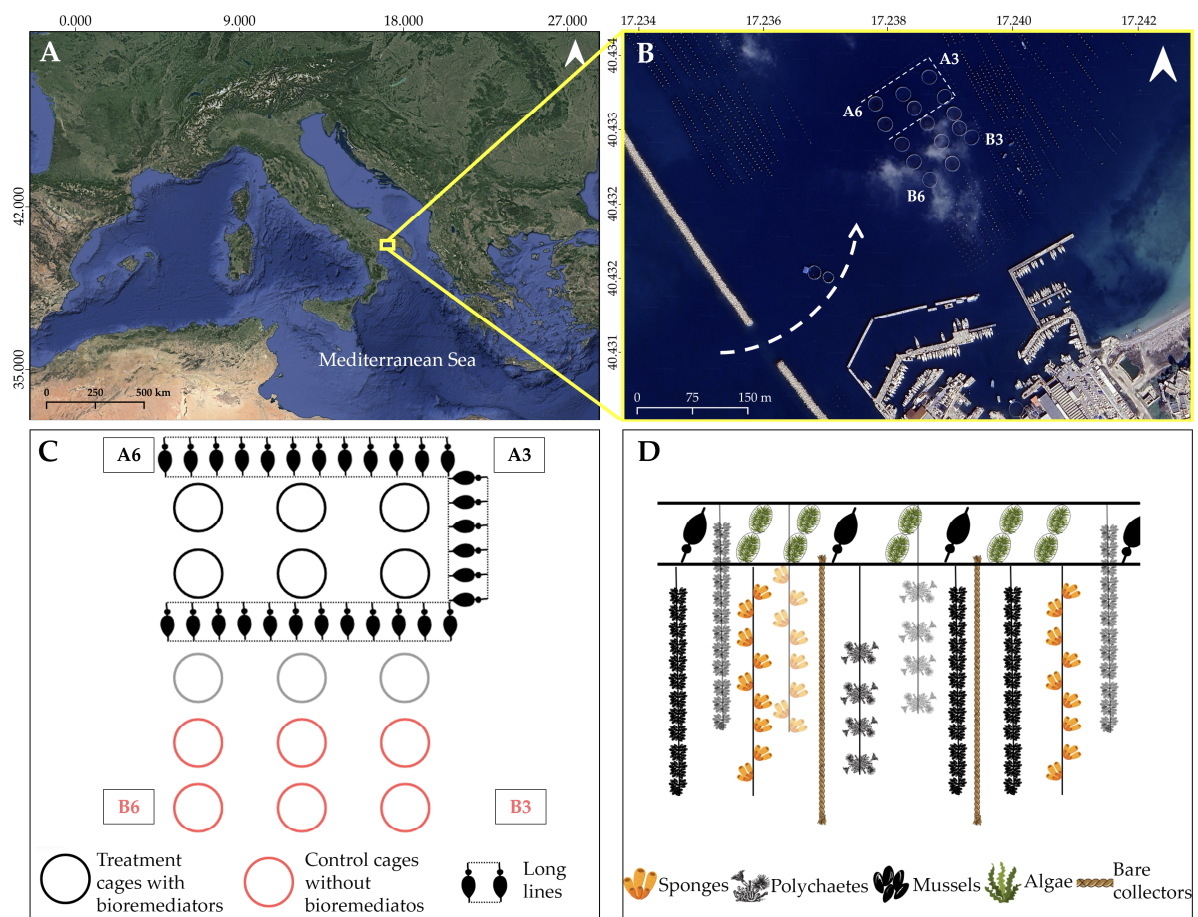
Studies have shown that IMTA systems can help restore mariculture environments and enhance biodiversity by leveraging biofouling organisms to mitigate the impact of organic waste [8,12,13]. In Mediterranean coastal ecosystems, where mariculture is both economically significant and ecologically impactful, understanding how these activities influence the surrounding environment is essential [14]. The shift in biomass and production of macro-zoobenthos in response to organic enrichment underscores the importance of understanding the recovery potential of benthic communities in mariculture-impacted areas [6]. The structure of benthic assemblages is in fact an effective indicator of the health status of coastal marine environments [15–17], and has been extensively used to assess the impact of aquaculture [18–22], as well as to detect their recovery after the ending of the activity [23]. Despite the recognized importance of hard-bottom benthic assemblages as indicators of environmental health [24], research on environmental health evaluation has primarily focused on soft-bottom communities affected by mariculture [18–22,25,26], while the potential effects of Integrated Multi-Trophic Aquaculture (IMTA) on hard-bottom benthic assemblages have not been thoroughly examined in the field [6,27,28]. Indeed, research to date has primarily focused on organic matter dynamics, suggesting that IMTA systems help maintain water quality [29,30]. However, recent studies have begun to reveal the significant influence of IMTA on soft-bottom benthic communities, particularly in terms of enhancing ecosystem health and resilience [31,32]. Such field investigations have shown that transitioning to an IMTA system can induce substantial changes in soft-bottom macro-zoobenthic communities, often referred to as the “biological memory” of the ecosystem. Evaluations of benthic biological indices (e.g., AMBI, MAMBI) within IMTA systems have demonstrated marked improvements in environmental conditions, including enriched species composition and higher ecological quality status [25,26].

As a complement to the studies conducted so far, which have primarily focused on soft-bottom benthic communities, this study aims to investigate the response of hard-bottom macro-zoobenthic communities to the conversion of a traditional mariculture plant into an IMTA system at the Maricoltura Mar Grande (MMG) site. By examining changes in hard-bottom macro-zoobenthic assemblages over a four-year period, this research provides critical insights into the environmental implications of transitioning to IMTA and its potential to enhance biodiversity and ecosystem sustainability in mariculture-impacted areas.

## 2. Material and Methods

### 2.1. Study Area

The study area is situated along the southwest coast of the Mar Grande of Taranto ( $40^{\circ}25'56''$  N;  $17^{\circ}14'19''$  E) in the Northern Ionian Sea. Together with the Mar Piccolo, this region forms one of the most significant coastal marine ecosystems along the Apulian coast in southern Italy (Figure 1). The Mar Grande of Taranto is a semi-enclosed basin connected to the Gulf of Taranto via three artificial dams. This area experiences significant anthropogenic pressures, including a commercial port in the northern section, a military harbor in the southern section, four tourist harbors located in the northeast and southern regions, the urbanized expanse of Taranto to the east, and numerous mussel farming facilities distributed throughout.



**Figure 1.** (A) Map of the study site; (B) location of the Maricoltura Mar Grande (MMG) facility; the white dotted line indicates the main current direction. (C) Schematic representation of the experimental area: The treatment area includes fish cages (black circles) equipped with long lines containing bioremediators, while the control area consists of fish cages (red circles) without bioremediators. Sampling points for the hard substrate, situated at the corners of the facility, are labeled A3, A6, B3, and B6. (D) Schematic depiction of the long line with bioremediators used in the IMTA system.

The present study was conducted at the “Maricoltura Mar Grande” (MMG) facility, which hosts the IMTA (Integrated Multi-Trophic Aquaculture) system as part of the EU REMEDIA Life project (LIFE16 ENV/IT/000343). The aquaculture facility, spanning an area of 0.06 Km<sup>2</sup> and situated approximately 600 m from the coast in a semi-confined section of the Mar Grande, produces about 100 tons of fish per year. It consists of 15 floating cages (Ø 22 m) with depths ranging from 7 to 12 m, used for farming European seabass *Dicentrarchus labrax* (Linnaeus, 1758) and sea bream *Sparus aurata* Linnaeus, 1758.

Since 2019, half of the MMG facility has been converted into an innovative IMTA plant, incorporating bioremediator organisms, such as sponges, polychaetes, bivalves, and macroalgae. A key innovation of this IMTA system is the use of artificial vertical collectors suspended in the water column, designed both for rearing bioremediators and promoting the natural settlement of extractive sessile macroinvertebrates. This novel approach enhances the efficiency of extractive species in improving environmental quality, aligning with the goals of sustainable aquaculture and ecosystem restoration [33].

The ex-ante analysis conducted in 2018, which included hydrodynamic measurements, revealed key characteristics of the area and informed the placement of bioremediator structures. Surface currents were observed to flow from northeast to southwest at approximately 3 cm s<sup>-1</sup>, while bottom currents moved in the opposite direction, from southwest to northeast, at a slower speed of around 1.3 cm s<sup>-1</sup> [33]. This analysis identified the most impacted area of the plant [14] and enabled the choice of the dislocation of the bioremediators' structure; so, the MMG plant was subdivided into two sectors: a treatment area, equipped with bioremediators, and a control area, without bioremediators. In the treatment area, three long lines (LLA, LLB, LLC) with bioremediators were associated with six fish floating cages (Figure 1). These long lines incorporated vertical collectors for invertebrate farming and horizontal collectors for macroalgae cultivation [33]. The long lines were supported by buoys to avoid the sinking of the structure due to the biomass growth (Figure 1). Each long line consisted of a series of floating buoys connected by double ropes. The space between two consecutive buoys formed a breeding “chamber” (16 chambers in LLA, 8 in LLB, and 12 in LLC), designed to house modules for bioremediators' rearing. These modules included vertical collectors for sponges, polychaetes, and bivalves, while macroalgae were positioned horizontally near the surface. Additionally, vertical bare collectors, made of 10 m long coconut fiber ropes (2 cm wide), were installed across all long lines to support the natural settlement of fouling species [12, see [33] for a detailed description of the IMTA plant]. The bioremediating organisms reared included the tube worm *Sabella spallanzanii* (Gmelin, 1791), the mussel *Mytilus galloprovincialis* (Lamarck, 1819), and four sponge species (*Sarcotragus spinosulus* (Schmidt, 1862), *Aplysina aerophoba* (Nardo, 1833), *Geodia cydonium* (Linnaeus, 1767), and *Hymeniacidon perlevis* (Montagu, 1814)), as well as two seaweed species (*Chaetomorpha linum* (O.F. Müller) Kützinger and *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva) [33–35].

These organisms were suspended on the long lines during different seasons within three rearing cycles. During each rearing cycle, a total of at least 540 vertical collectors for invertebrate bioremediators and 186 horizontal sockets for macroalgae were deployed [33].

## 2.2. Environmental Parameters

In order to characterize the experimental area, at each sampling time, physical-chemical parameters such as temperature (°C), pH, dissolved oxygen (DO, ppm), and salinity (PSU) were measured in triplicate in the middle of the experimental area at a depth of about 50 cm using HANNA instruments' multiparametric probe HI98194.



### 2.3. Biological Sampling

The hard-bottom macro-zoobenthic community was investigated on permanently submerged artificial hard substrates, including iron chains and concrete anchoring blocks, placed on unvegetated muddy sediment at a depth of 12 m.

In order to evaluate the changes in the macro-zoobenthic community, three replicates were collected twice a year (winter and summer) from 2018 (ex-ante analysis) to 2020 in four stations located at the four corners of the experimental area (two in the treatment area: A3, A6; two in the control area: B3, B6). Additional sampling was conducted during the summer 2021, for a total of 84 of samples.

The biological samples were collected with a semiquantitative methodology, standardized in similar research [36,37], which consisted of scraping off three replicates (R1, R2, R3) of 20 cm<sup>2</sup> at each station. The samples were collected by scuba divers at 12 m depth using direct hand collection. To ensure motile organisms were adequately captured, the samples were placed directly into plastic bags underwater. This method minimized the risk of losing vagile organisms during collection. The plastic bags were securely sealed while still submerged, ensuring a representative sample of the community was preserved. The samples collected were sorted and identified in the laboratory at the most detailed level possible by specialists of different groups. The nomenclature adopted is reported in the relevant updated check list of the World Register of Marine Species [38].

The abundance of macroinvertebrates, ranging from “absent” to “extremely abundant”, was assessed using a qualitative scale, later converted into a numerical scale. The macroinvertebrates were evaluated using a six-class abundance scale, modified by [39]. The scale includes:

0. Absence.
1. Presence of isolated individuals or colonies.
2. A few individuals or colonies.
3. Numerous individuals or colonies.
4. Very numerous individuals or colonies.
5. Overwhelming presence (dominance).

This method is particularly useful when counting individual/colonial organisms like sponges and bryozoans, where direct enumeration is challenging (Warwick, 1993). The qualitative values were converted into conventional numerical values (1–10–50–100–500) for quantitative analysis. This approach, used in previous studies [37,40], facilitates the comparison of taxa that differ significantly in size, growth form, biomass, physiology, and ecology. The resulting numerical data were employed for further univariate and multivariate analyses.

For each macroinvertebrate species identified, the habitus (vagile, sessile) and the alien status were annotated. Moreover, taking into account that species interactions are fundamental to ecosystem functions and play a crucial role in understanding ecosystem changes [41], feeding guilds of the macro-zoobenthic invertebrates found have been considered [42–54]. The different feeding guilds listed were Oms—Omnivores, Hes—Herbivores, Gs—Grazers, Ps—Predators, FFs—Filter Feeders, DFs—Deposit Feeders, Ds—Detritivores, Cs—Carnivores.

### 2.4. Biodiversity Indices and Statistical Analysis

Univariate descriptors were calculated for each site and station to characterize the macro-zoobenthic communities over the years and across different stations. Community indices, such as taxa richness (total number of taxa), diversity [55], evenness (H/S and J; [56]), and the Margalef species richness [57], were calculated for the entire benthic community using converted abundance scale values.

In addition, as estimators of taxonomic diversity, we used the average taxonomic distinctness ( $\Delta+$ ) as well as the variation in taxonomic distinctness index ( $\Delta+$ ) [58]. The average taxonomic distinctness ( $\Delta+$ ) was estimated from node-based phylogenetic trees and was calculated using presence–absence data. Funnel-shaped charts and biplot graphs were constructed for the visual presentation of results [59].

Permutation analysis of variance, PERMANOVA [60], was performed to test for differences in the composition of macrofouling assemblages in relation to two factors: season\_year (SE; seven levels: S\_18, W\_18, S\_19, W\_19, S\_20, W\_20, S\_21) and site (ST; four levels: A3, A6, B3, B6). Square-root-transformed data in a Bray–Curtis similarity matrix with 9,999 permutations were used to perform the analyses. If it was impossible to obtain enough permutations for PERMANOVA analysis, the reference  $p$  was obtained using a permutation simulation test (Monte Carlo test). When significant interactions were detected ( $p < 0.05$ ), differences among assemblages across factor levels were identified with post hoc pairwise comparison based on the significant value of the PERMANOVA/Monte Carlo tests.

Principal Coordinates Analysis (PCO), based on Bray–Curtis distance, was used to illustrate how the macrofouling assemblages vary between control sites and treated sites across seasons and years.

The SIMPER (similarity percentage breakdown, [61]) analysis was then applied to obtain the contribution of each species to the average similarity within the groups identified with the clustering procedure. The cutoff criterion for identification was set at 70%.

PCO and SIMPER analysis were applied on square-root-transformed abundance data.

The analyses were carried out using PRIMER 6 with PERMANOVA + software [62].

### 3. Results

#### 3.1. Environmental Parameters

The seawater parameters registered across different seasons (winter—W, summer—S) for the years 2018 to 2021 are given in Table 1. Overall, the seasonal variations showed that summer months typically exhibit higher temperatures, higher salinity, and lower dissolved oxygen levels. The winter temperatures ranged from 14.4 °C to 17.2 °C, while the summer temperatures ranged from 26.4 °C to 28.2 °C, showing a predictable seasonal variation. The pH values were generally around 7.1 to 8.1. Summer seasons tended to exhibit slightly higher pH levels compared to winter, indicating more alkaline conditions during the warmer months. Dissolved oxygen (DO, ppm) levels were higher in winter (ranging from 7.6 to 7.8 ppm) and decreased in summer (ranging from 5.3 to 6.9 ppm), likely due to increased temperature affecting oxygen solubility. Salinity showed some seasonal fluctuations, with slightly higher values typically in summer and slightly lower values in winter. Over the years, a slight decreasing trend was observed from 38.8 PSU to 34.2 PSU during winter months.

**Table 1.** Physicochemical variables values (mean  $\pm$  standard deviation) during the study periods measured in the experimental area.

	Season	Temperature (°C)	pH	DO (ppm)	Salinity (PSU)
2018	W	17.2 $\pm$ 0.9	7.1 $\pm$ 0.1	7.8 $\pm$ 0.1	38.8 $\pm$ 0.2
	S	26.4 $\pm$ 0.8	8.0 $\pm$ 0.2	6.9 $\pm$ 0.2	37.7 $\pm$ 0.8
2019	W	16.4 $\pm$ 0.5	7.9 $\pm$ 0.1	7.6 $\pm$ 0.4	35.6 $\pm$ 0.7
	S	27.2 $\pm$ 0.2	8.1 $\pm$ 0.1	6.6 $\pm$ 0.2	36.6 $\pm$ 0.4
2020	W	14.4 $\pm$ 0.5	8.0 $\pm$ 0.2	7.8 $\pm$ 0.4	34.2 $\pm$ 1.8
	S	28.2 $\pm$ 0.2	8.1 $\pm$ 0.1	5.7 $\pm$ 0.4	37.4 $\pm$ 0.7
2021	S	27.9 $\pm$ 0.7	8.1 $\pm$ 0.1	5.3 $\pm$ 0.2	36.7 $\pm$ 0.3

### 3.2. Descriptive Analysis

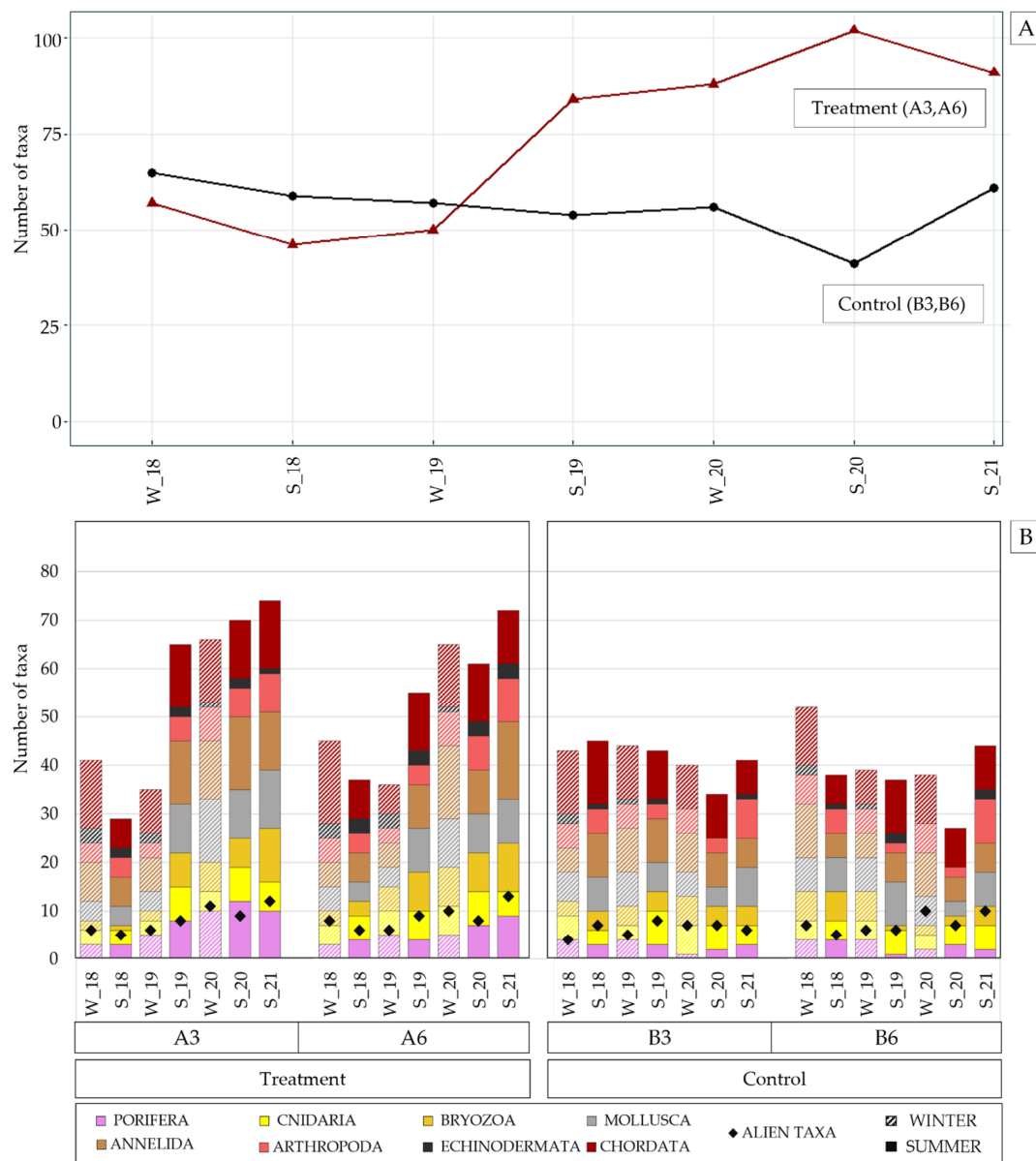
Overall, a total of 130 taxa were identified on the hard substrates under the fish cages during the study period, 122 of them identified at species level. The most prevalent taxa belong to Tunicata and Annelida, with 26 and 24 taxa, respectively, followed by Mollusca and Porifera, with 19 and 20 taxa, respectively. Crustacea, Bryozoa, Cnidaria, and Echinodermata were less represented (Table S1 Supplementary Materials). The number of taxa observed on hard substrates under the fish cages increased from 83 in 2018 to 104 in 2021. Although the overall increase may not appear dramatic, the taxa richness demonstrated a steady upward trend within the treatment area, whereas the number of taxa in the control area remained relatively constant throughout the study period (Figure 2A). The increase in the treatment area (stations A3, A6) was primarily driven by species observed in the summer samples, with the number of taxa increasing from 46 in 2018 to 84 in 2019, reaching 102 in 2020, and 91 in 2021 (Figure 2A). By contrast, the control area (stations B3, B6) showed only minor fluctuations over time, with a notable decrease during the summer of 2020 (Figure 2A). In 2019 summer samples from the treatment area, groups such as annelids, bryozoans, mollusks, poriferans, and ascidians exhibited at least a doubling in species richness with respect to that found in 2018 summer samples (Figure 2B). Additionally, an increase in taxa was also recorded in the winter samples of the treatment area, rising from 57 taxa in 2018 to 88 in 2020 (Figure 2B).

Overall, 19 alien species were recorded on the hard substrates under the fish cages, accounting for 14.6% of the total species observed. The most represented taxa were Chordata (Tunicata) and Annelida (seven and six species, respectively), while Bryozoa, Crustacea (two species each), Mollusca, and Porifera were less represented (one species each). *Hydroides elegans* (Haswell, 1883), *Paracerceis sculpta* (Holmes, 1904), and *Amathia verticillata* (delle Chiaje, 1822) were exclusively found in the treatment stations (A3, A6) (Table S1 Supplementary Material). The treatment stations exhibited a slight increase in the number of alien species over time, while the values at control ones showed a relatively stable trend (Figure 2B). When alien species were considered a percentage of the total species recorded in each station, the control area (particularly station B6) showed a marked rise around 2020. In contrast, the treatment area (A3 and A6) showed more stable trends with fewer pronounced peaks. These findings suggest potential differences in alien species settlement dynamics between the treatment and control areas, reasonably influenced by differing environmental conditions established in each area.

Sessile species consistently made up the majority component, indicating a larger representation compared to mobile fauna found (Figure 3). The vagile taxa were mainly Crustacea (10 taxa) and Annelida (6 species). In the summer of 2021, the highest number of vagile species was recorded at station A6 (12 taxa, representing 16.7% of the total taxa), nine of which were absent in the 2018 sampling. In the control stations, the number of vagile species was lower, with no increase over time.

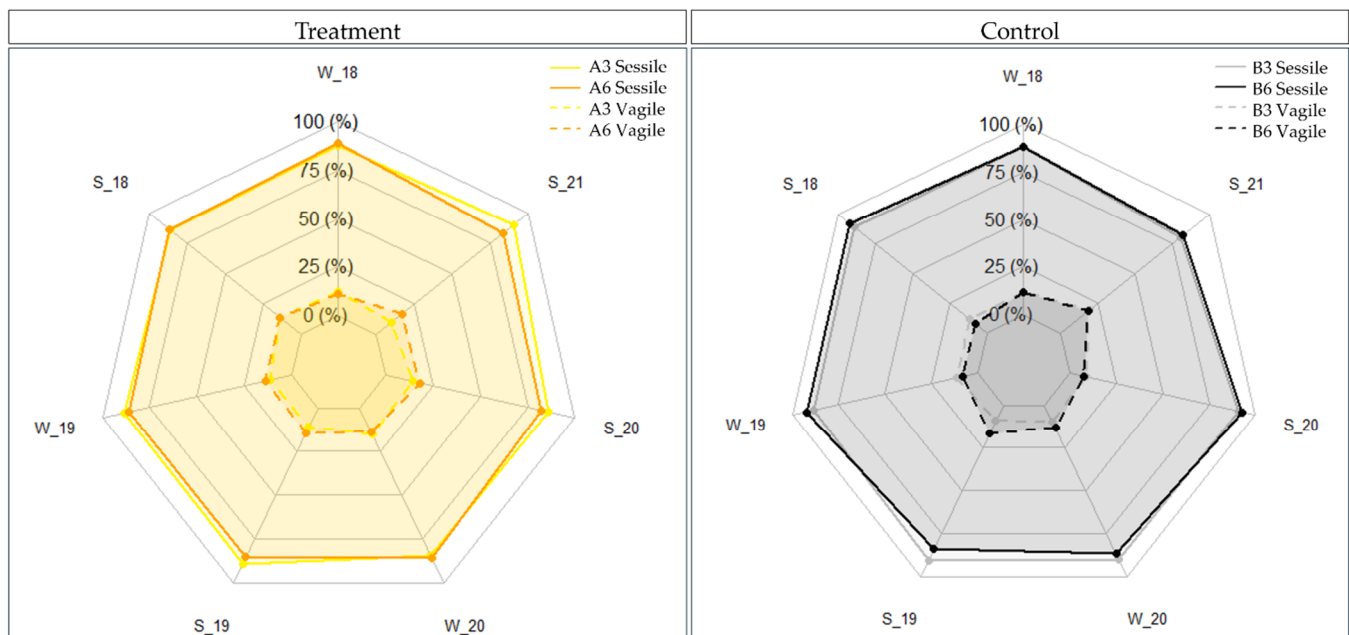
At the first observations in 2018, prior to the installation of the IMTA system, the artificial hard substrates located under the cages were sparsely inhabited by macro-zoobenthic organisms, with significant portions of the substrate covered in sediments. By the end of the observation period in 2021, a revitalized and more diverse macro-zoobenthic community was observed in the treatment area, whereas no substantial changes were observed in the control area. Sessile species under the cages were mainly composed of typical fouling communities found in confined or semi-confined environments, supplemented by species commonly found on hard substrates in less transient communities, such as poriferans. In this regard, it is noteworthy that the hard substrates of the treatment stations at the beginning of the observations (2018) hosted only four species of sponges (*Hymeniacidon perlevis* (Montagu, 1814), *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, *Tethya meloni*

Corriero, Gadaleta & Bavestrello, 2015, and *Clathrina coriacea* (Montagu, 1814)). However, in the last sampling (summer 2021), in addition to the four initial species, eight new taxa were recorded (*Aplysilla rosea* (Barrois, 1876), *Dysidea incrustans* (Schmidt, 1862), *Haliclona mediterranea* Griessinger, 1971, *Leucandra crambessa* Haeckel, 1872, *Oceanapia isodictyiformis* (Carter, 1882), *Sarcotragus spinosulus* Schmidt, 1862, *Sycon ciliatum* (Fabricius, 1780), and *Sycon* sp.), bringing the total number of sponge taxa to 12. At the end of the observations, in the treatment stations, annelids, molluscs and bryozoans were the main groups showing a noticeable increase in number of species (Figure 2). The increase in species richness can be partially attributed to vagile associated fauna, which displayed a less diverse taxonomic pattern compared to sessile fauna. Exceptions, such as *Naineris setosa* (Verrill, 1900), indicate that the vagile fauna present is largely atypical of confined environments. Overall, the balance between sessile and mobile fauna has remained relatively stable over the years, with minor fluctuations observed in both the treatment and control areas (Figure 3).



**Figure 2.** Total number of taxa recorded during the study period (2018–2021). (A) Taxa richness in the treatment (red line) and in the control stations (black line); (B) distribution of taxa within taxonomic groups. The black diamonds indicate the number of recorded alien species (W—winter, S—summer).





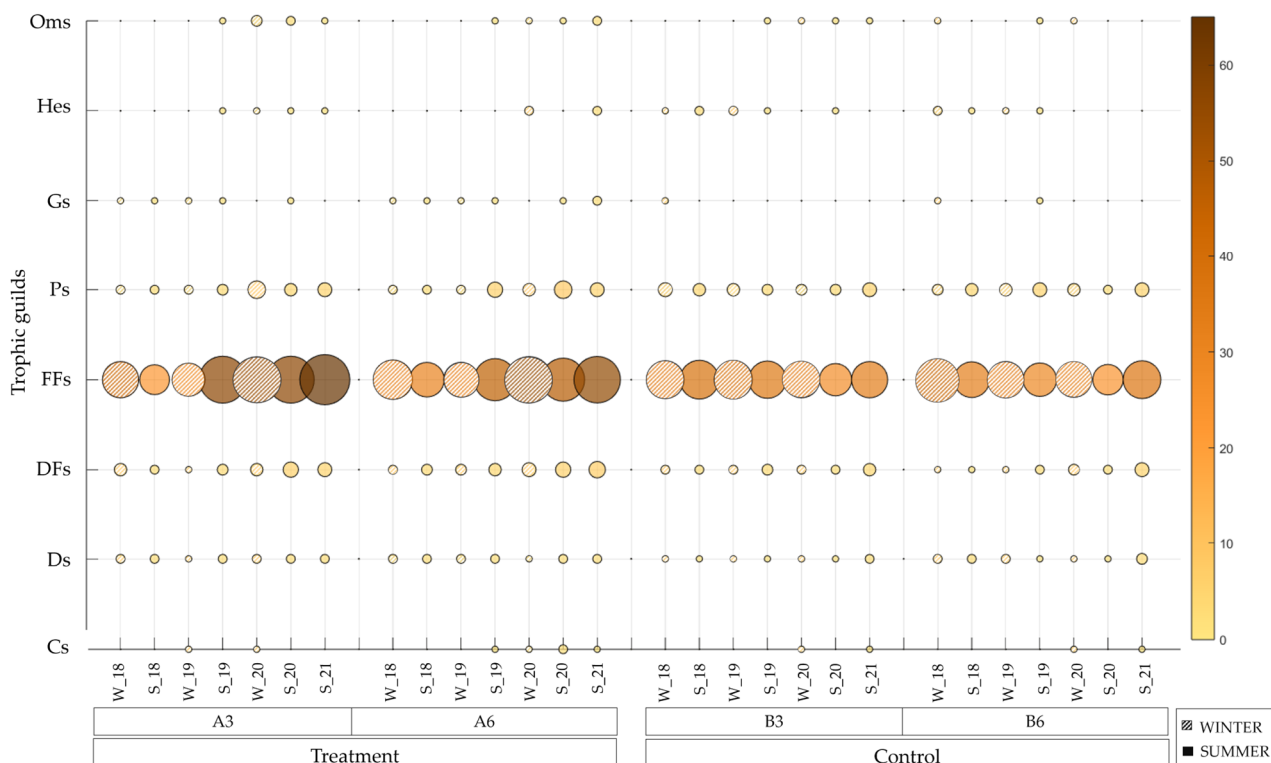
**Figure 3.** Radar plots visualization of sessile and vagile macro-zoobenthic taxa (%) recorded in the treatment area (left, stations A3 and A6) and control area (right, stations B3 and B6). (W—winter, S—summer).

The bubble plot in Figure 4 depicted the abundance of different feeding guilds over time, spanning multiple years (2018 to 2021) across different sampling seasons (W = winter, S = summer). The size and color intensity of the bubbles corresponded to the abundance of each feeding guild. Larger and darker-colored bubbles indicated higher abundance for that guild. The temporal dynamics and distribution of feeding guilds across stations were clearly represented, emphasizing shifts in community composition mainly involving the Filter Feeders (FFs). In the studied community, FFs were prominently represented, with larger bubbles across the entire time frame, suggesting their consistent prevalence. Other feeding guilds like Omnivores (Oms), Herbivores (Hes), Grazers (Gs) had relatively smaller bubbles, indicating lower relative abundance or less representation. Seasonal and interannual variation was evident, particularly for FFs, as slight changes in bubble size and intensity were observable between sampling points over the years. Focusing on the difference between the first (2018) and last (2021) summer of sampling, a notable increase in Predators (Ps) and Deposit Feeders (DFs) was observed at both treatment areas (A3, A6). Additionally, Omnivores (Oms), which were absent in the summer of 2018, emerged by the final sampling period. Finally, Filter-Feeding (FF) taxa exhibit a substantial increase at both stations, with the number at the A3 station growing from 23 in 2018 to 65 taxa in 2021, and increasing from 31 to 56 taxa at the A6 station.

### 3.3. Biodiversity Indices and Statistical Analysis

In Figure 5, the different biodiversity indices measured at four stations (A3, A6, B3, B6) over winter (W) and summer (S) from 2018 to 2021 are represented. The treatment area (stations A3 and A6) generally showed increases in biodiversity indices over the study period, particularly during summer months, suggesting an improvement in diversity and evenness. In contrast, the control area (stations B3 and B6) exhibited relatively stable values with less variability over time. The Shannon Index ( $H'$ ) reflected species diversity that, across the observed period, showed an overall increasing trend at stations A3 and A6 (treatment area), particularly noticeable in the winter samples of 2020. In contrast, the control area at stations B3 and B6 showed relatively stable diversity levels with minor

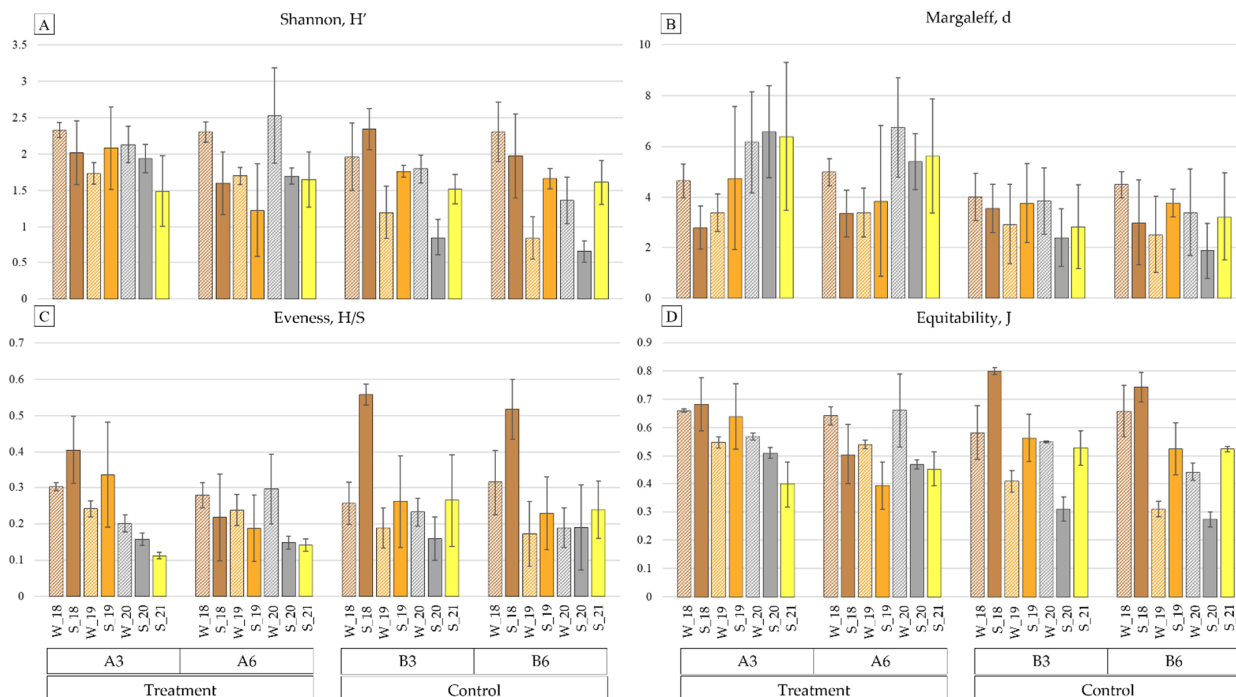
fluctuations over time. Also, the Margalef Index displayed an increasing trend at stations A3 and A6 (treatment area), particularly during the winter and summer of 2020, indicating a growth in species richness. The control area (stations B3 and B6) showed minor changes in the Margalef Index and remained relatively stable over the years. The measures of the species distribution obtained with the Evenness Index (H/S) showed a considerable variability, with station B6 exhibiting higher evenness in the early years (2018). The treatment area (stations A3 and A6) showed a gradual decrease in evenness over time, particularly during later summers. A gradual reduction in the Evenness Index at a station over time indicates that the biological community is becoming less balanced. In other words, certain species are becoming more dominant than others, leading to an unequal distribution of individuals among the species present. While this trend is generally considered negative, in our case, it highlights the significant shift experienced by the zoobenthic community in the treatment stations due to the installation of the IMTA system. The Equitability Index (J) measures the evenness of species distribution but operates on a different scale than H/S. Similar to H/S, J displayed relatively stable values over time in treatment station A6, whereas it fluctuated for A3, with summer of 2020 exhibiting lower equitability. At the control sites (B3 and B6), equitability at B3 consistently remained lower than at B6, indicating a less uniform species distribution at the former control site.



**Figure 4.** Bubble plot displaying trophic guilds across the treatment area (stations A3, A6) and control area (stations B3, B6) during the studied period (Oms—Omnivores, Hes—Herbivores, Gs—Grazers, Ps—Predators, FFs—Filter Feeders, DFs—Deposit Feeders, Ds—Detritivores, Cs—Carnivores; W—winter, S—summer).

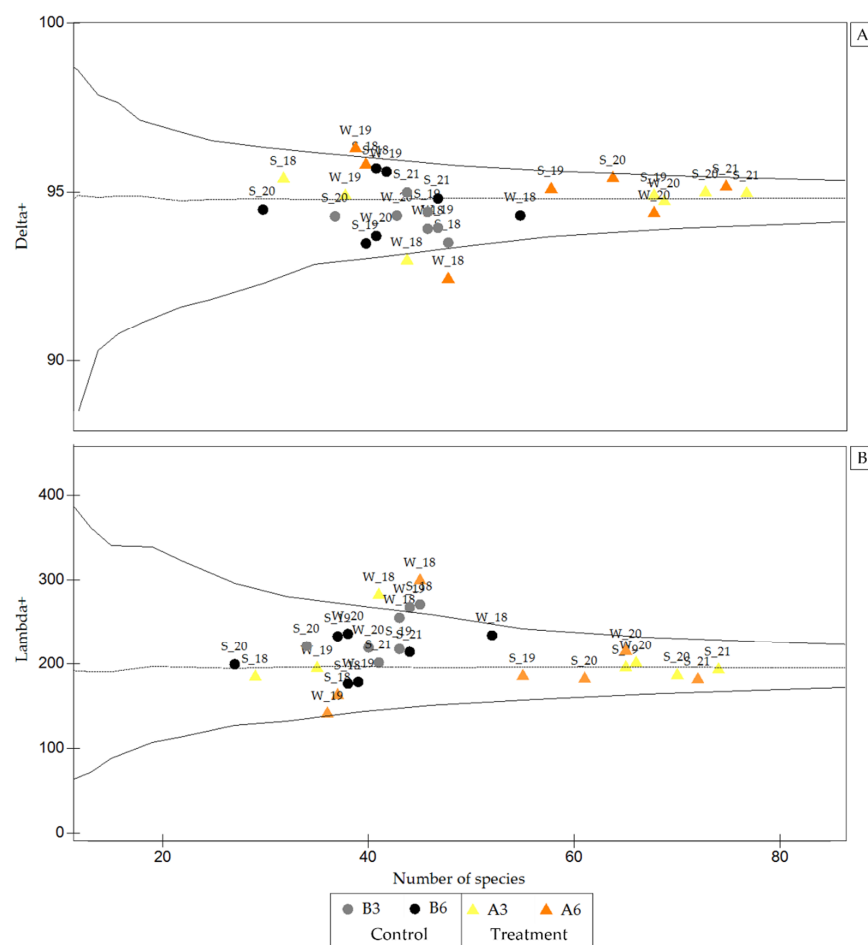
The average taxonomic distinctness (delta+) obtained in the treatment and control area (stations A3 and A6, and B3 and B6, respectively) over time is reported in Figure 6A. Delta+ measures the average taxonomic range of species in a sample, offering insight into a community structure beyond simple species counts, such as phylogenetic diversity and how distinct the species are from each other in terms of taxonomy. Most of the samples clustered close to the expected mean of delta+ (horizontal dashed line), indicating that the

taxonomic structure was mostly consistent with what would be expected based on random assemblages of similar size. Some points (A6 in W\_18 e W\_19; A3 in W\_18) fall closer to the lower confidence limits (solid lines). At site A3, during the first summer in 2018, delta+ was high (95.4) but with moderate statistical significance (59.1). Starting from winter 2019, delta+ values ranged between 94.73 and 95.00, showing high statistical significance (values higher than 70%). As for the control sites, at B3, all delta+ values were below the expected mean of delta+, ranging from 93.52 (S\_18) to 94.43 (S\_19), except for summer 2021, which showed a value of 95. At site B6, the highest delta+ values were observed in summer 2018 and summer 2019, with summer 2021 showing the expected value. Regarding lambda+ (Figure 6B), at site A3, the lowest values were observed in summer 2018, 2020, and 2021. Also, at station A6, the lowest lambda+ values were observed in the summer 2020 and 2021, both with moderate statistical significance (with values ranging from 40 to 50%). By contrast, winter 2018 and summer 2018 showed higher lambda+ values (298.92 and 162.30), with lower statistical significance (0.2 and 28.8%, respectively). At control site B3, lambda+ values were generally high, suggesting a less distinct taxonomic structure. Finally, at site B6, the lowest lambda+ values were observed in summer 2018 and winter 2019, with moderate statistical significance (64.1 and 65.7%, respectively) (Table S2, Supplementary Materials).



**Figure 5.** Biodiversity indices. Mean values ( $\pm$  standard deviation) of the Shannon diversity index (A), Margaleff (B), Evenness (C) and Equitability (D) of macro-zoobenthic communities across different stations over the years (W—winter, S—summer).

The multivariate PERMANOVA test (Table 2) showed that macrofouling communities were significantly affected by the factors site (ST) and season\_year (SE). Furthermore, the interaction between these two factors also significantly affected the composition of the communities. Post hoc pairwise comparisons (Table 2) showed that, overall, in summer 2018, there was a marginally significant difference between stations, and no significant differences between A3 and B3, or between B3 and B6. At the end of the experiment, in summer 2021, significant differences were observed when comparing treated stations and control stations, whereas no significant differences were detected between A3 and A6, or between B3 and B6 (Table 2).



**Figure 6.** Funnel plot of the average taxonomic distinctness ( $\Delta+$ ; (A)) and variance in taxonomic distinctness ( $\Lambda+$ ; (B)) for treatment stations (A3, A6) and control stations (B3, B6). In both graphs, the global average is shown as a dotted line and the 95% confidence interval as a continuous curve with 999 permutations of randomized species pairings from the whole species list (W—winter, S—summer).

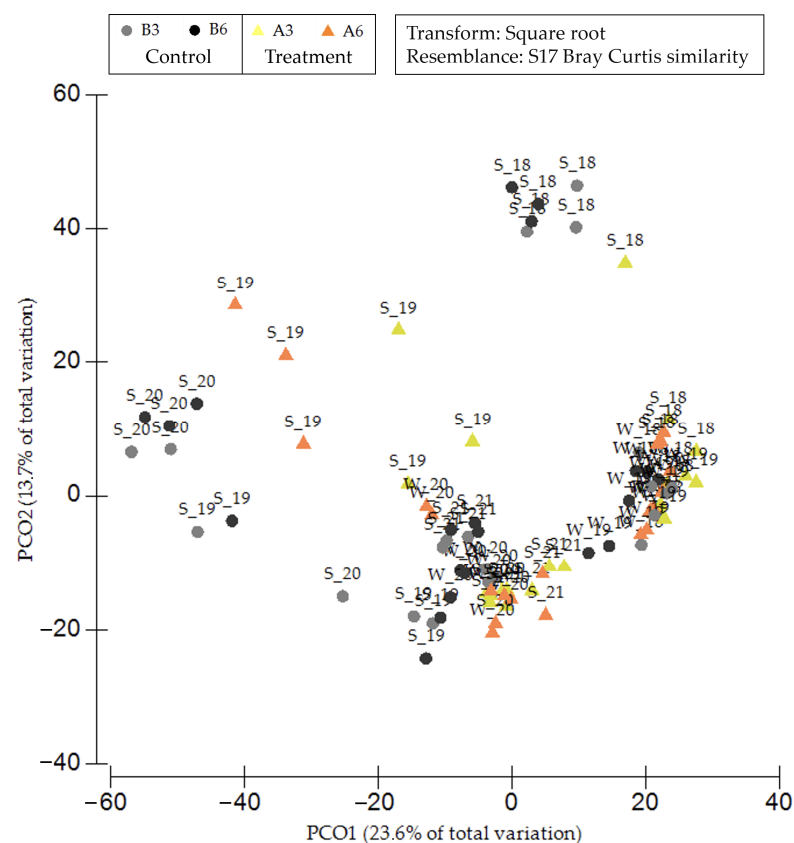
**Table 2.** Results from permutation analysis of variance (PERMANOVA) analysis. \* =  $0.01 < p(\text{MC}) < 0.05$ ; \*\* =  $p(\text{MC}) < 0.01$ ; ns = not significant. ST = site; SE = season\_year; W = winter; S = summer; A3, A6 = treatment stations; B3, B6 = control stations.

PERMANOVA							
Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
ST	3	13,884	4628	88.868	0.0001	9907	**
SE	6	67,432	11,239	21.581	0.0001	9865	**
STxSE	18	52,941	2941.2	56.478	0.0001	9774	**
Res	56	29,163	520.77				
Total	83	$1.63 \times 10^5$					
Pairwise test							
P (MC)							
	W_18	S_18	W_19	S_19	W_20	S_20	S_21
A3, A6	**	*	**	ns	*	**	ns
A3, B3	*	ns	*	ns	**	**	**
A3, B6	**	*	*	*	**	**	*
A6, B3	*	*	**	*	**	**	**
A6, B6	**	*	**	*	**	**	**
B3, B6	*	ns	ns	ns	*	ns	ns

The Principal Coordinates Analysis (PCO) revealed that the first two axes explained 23.6% and 13.7% of the variance, respectively, in macro-zoobenthic assemblages across



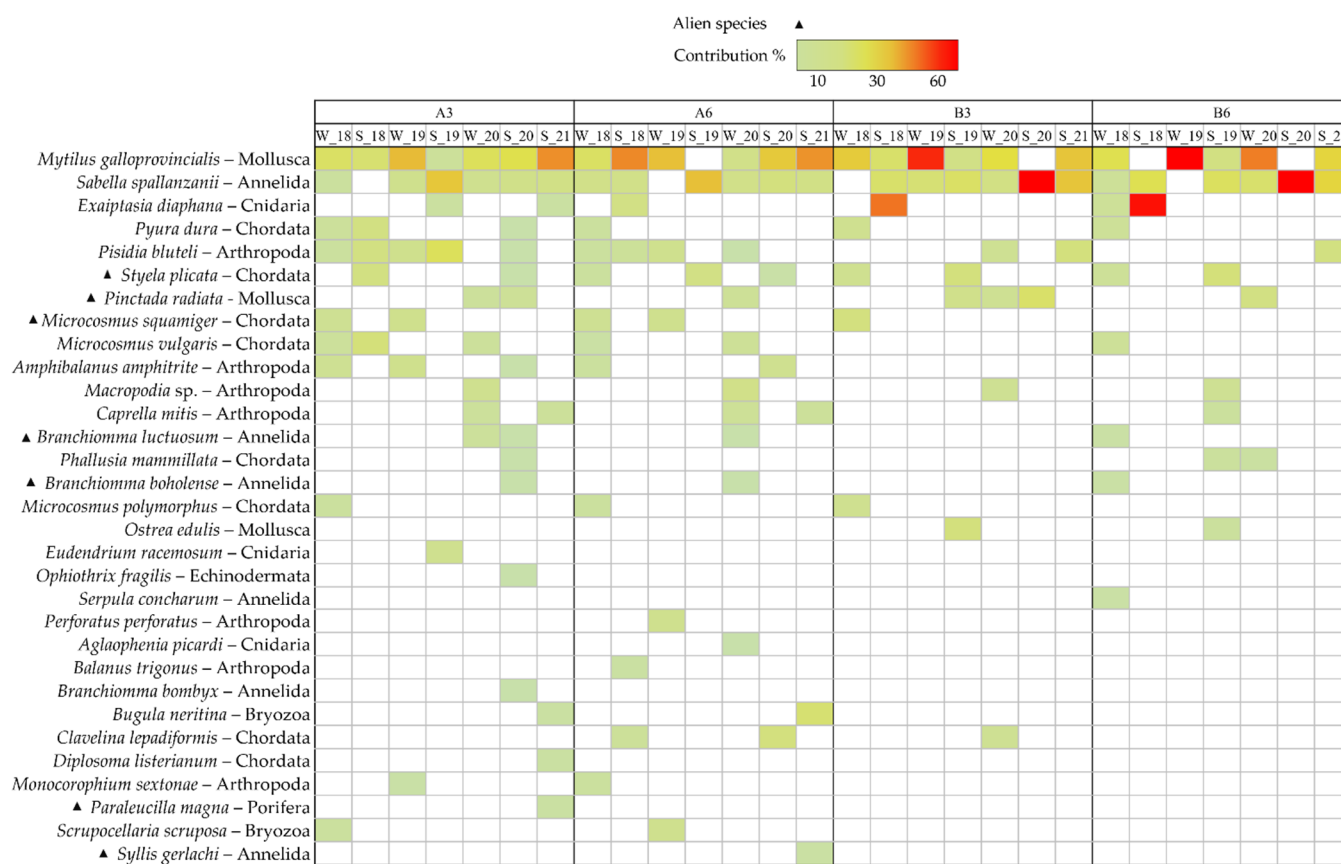
years and seasons at each site. Over time, the community composition at the treatment stations (A3, A6) shifted, creating a new grouping that encompassed assemblages from summer 2020, summer 2021, and winter 2020, highlighting the influence of the IMTA system installation on community dynamics. In contrast, control stations (B3, B6) tended to cluster more tightly, indicating greater stability and higher similarity in community composition across seasons and years. The treatment stations (A3, A6) exhibited a broader spread in the ordination space, reflecting greater variability in zoobenthic communities, likely driven by environmental changes associated with the IMTA system. Notably, points from Summer 2019 (S\_19) and Summer 2020 (S\_20) at the treatment stations appeared distinctly separated from other years, suggesting significant changes in species composition during those periods (Figure 7).



**Figure 7.** Principal Coordinates Analysis (PCO) plot of macro-zoobenthic communities from 2018 to 2021 at each station (A3, A6, B3 and B6).

The SIMPER (similarity percentage) analysis identified the contribution of different taxa to the similarity among samples, with a color-coded heatmap illustrating the percentage contribution (Figure 8). Overall, the analysis revealed that 30 species out of the 130 surveyed taxa account for over 70% of the cumulative contribution to the hard-substrate macro-zoobenthic communities, including seven alien species: *Styela plicata* (Lesueur, 1823), *Pinctada radiata* (Leach, 1814), *Microcosmus squamiger* Michaelsen, 1927, *Branchiomma luctuosum* (Grube, 1870), *B. bohollense* (Grube, 1878), *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, and *Syllis gerlachi* (Hartmann-Schröder, 1960). Notably, only a few dominant species significantly influenced community composition at each station over time, ranging from a minimum of 1 to a maximum of 12 species (Figure 8, Table S3 Supplementary Material). Shifts in the dominance and contribution of these taxa reflected changes in community structure and reasonably suggested responses to IMTA installation influencing species dynamics at each station. The 70% cut-off ensured that the analysis

focuses on the key taxa driving similarities among samples. The analysis showed that *Mytilus galloprovincialis* Lamarck, 1819, and *Sabella spallanzanii* (Gmelin, 1791) exhibited significant contributions throughout the entire observation period in both the treatment and control areas. The anemone *Exaiptasia diaphana* (Rapp, 1829) played a notable role in the control area (stations B3, B6) during the summer of 2018. Similarly, *Pisidia bluteli* (Risso, 1816) contributed significantly in the summer samples of 2018 and 2019, particularly in station A3, but its contribution ceased by 2021. In contrast, at the control sites, the contribution of *P. bluteli* became negligible or absent after winter 2020. Among the alien species, *M. squamiger* Michaelsen, 1927, and *P. radiata* (Leach, 1814) showed notable contributions. Overall, distinct patterns of dominant taxa emerged between the treatment and control areas: at stations A3 and A6, a higher number of species contributed to cumulative similarity, whereas at stations B3 and B6, fewer species dominated, contributing more significantly to cumulative similarity (Table S3, Supplementary Materials).



**Figure 8.** Results of SIMPER analysis showing percentage contributions of macrofaunal taxa that accounted for most of the similarity (70%).

#### 4. Discussion

This study, conducted as part of the REMEDIA Life project, demonstrates the positive effects of transforming a traditional Mediterranean mariculture site into an Integrated Multi-Trophic Aquaculture (IMTA) system on hard-bottom macro-zoobenthic communities. By examining changes in biodiversity, community composition, and trophic structure, our findings highlight the potential of IMTA systems to enhance environmental conditions and support diverse hard-bottom benthic communities. The increase in taxa richness from 83 in 2018 to 104 in 2021, especially tunicates, annelids, and mollusks, within the treatment area, coupled with the relatively stable species count in the control area, suggests that IMTA may play a key role in enhancing the biodiversity of hard-bottom communities. These findings align with previous research conducted at the same sites reporting biodiversity enhance-

ments in soft-bottom communities under the IMTA systems [25,26], thus emphasizing that biodiversity enhancement is a potential outcome of IMTA systems.

Future research should focus on scaling these findings across different environmental settings and exploring additional biotic interactions within IMTA framework. Overall, the findings presented are consistent with the hypothesis that the integration of extractive species in IMTA, able to mitigate nutrient load, can improve environmental conditions that support diverse benthic communities. In other words, the IMTA systems can enhance biodiversity and improve benthic community health by fostering diverse, resilient macro-zoobenthic assemblages, as well as on hard substrate, thus contributing to the growing evidence that IMTA offers a sustainable pathway for balancing production with environmental stewardship, particularly in Mediterranean mariculture ecosystems.

The environmental amelioration produced by the IMTA displacement in the Maricoltura Mar Grande site within the project REMEDIA Life was verified in previous investigations [8,25,26]. The role of suspension feeders as bioremediating organisms in influencing water column seston and mitigating fish farm waste was evaluated through the monitoring changes in soft-bottom communities beneath the cages over three years following the installation of the IMTA system [25] and by one year of monthly monitoring (from July 2020 to October 2021) of physicochemical and biochemical variables of the water column and organic matter (OM) in the sediment [8].

However, these earlier studies primarily focused on water column and soft-bottom communities [8,25,26]; our research expands this knowledge by exploring the hard-bottom substrates, which have often been overlooked in monitoring programs of mariculture facilities [6,27,28]. By directly comparing the pre- and post-IMTA conditions, we provide new evidence that the installation of bioremediating organisms on vertical collectors contributes significantly to biodiversity enhancement, ecological resilience, and habitat restoration.

We have shown how bioremediating organisms may function as underwater gardens, altering seston quality and availability while offering protection and food for the zooplankton community. As a result, the IMTA site exhibited a higher abundance of zooplankton individuals. Moreover, the findings of Borghese et al. [8] revealed that OM content in sediments beneath fish cages was significantly higher at the control site compared to the treatment site after IMTA installation, thus demonstrating the effectiveness of the considered IMTA system in mitigating the negative impact of organic matter release into the environment due to fish farming activities. The influence of bioremediating organisms on seston composition and concentration opened up mitigation as well as restoration scenarios. Lastly, the changes occurred as a whole: water column and substrates are reported in the work of Stabili et al. [26]. In such papers [25,26], the MMG plant revealed significant improvements in environmental conditions, particularly after the conversion of the fish farm to the IMTA system. The AMBI and M-AMBI indices indicated a clear improvement in the environmental status of treatment area (A), from “poor” to “good” conditions, which aligns with the observed changes in the hard-bottom zoobenthic community composition of the present research. The changes in the soft-bottom community, such as the decrease in opportunistic species like *Capitella capitata* and the increase in species indicating better environmental conditions, are directly related to the improvements in sediment quality and organic matter load. These changes in the sediment environment, as reflected by the AMBI and M-AMBI indices, provide a more complete picture of how the IMTA positively influenced the benthic ecosystem. However, the hard-bottom communities were generally neglected in these monitoring studies [6,27,28]. Only in the work of Arduini et al. [12] was a comparison of the two hard substrates within the REMEDIA Life IMTA plant (vertical collectors and hard substrates under the cages) shown, the last one characterized by a richer

community with an increase in biodiversity after the placement of vertical collectors. The changes that occurred at the hard-bottom substrates were mainly attributed to the decrease in particulate matter and sedimentation rate originating as wastes from the breeding cage, which were intercepted by the filter feeder community developed on the vertical collectors.

In the present study, the changes occurred in the hard-bottom macro-zoobenthic community under the fish cages were examined in detail. In 2018, prior to the installation of the IMTA system, the artificial hard substrates located under the cages were sparsely inhabited by macro-zoobenthic organisms, with significant portions of the substrate covered by sediments. By the end of the observation period in 2021, a revitalized and more diverse macro-zoobenthic community was observed in the treatment area, whereas no substantial changes were observed in the control area. The increase in biodiversity indices, including the Shannon and Margalef indices, over the study period for the treatment sites further supports the notion of a positive ecological impact of the bioremediators. The greater diversity and evenness, particularly during the summer months, underscore a revitalized and more resilient benthic environment, contrasting with the control sites, where little change in biodiversity metrics was observed, emphasizing that the ecological shifts were associated with the IMTA conversion rather than broader environmental trends. The presence and stabilization of sessile macrofauna, such as filter feeders, are particularly noteworthy. Filter Feeders are known to contribute significantly to nutrient cycling and water clarity, and their increased representation suggests that the IMTA system successfully enhances water quality. The observed doubling in Filter-Feeding taxa supports previous findings that IMTA systems improve nutrient retention and foster habitat complexity [12]. In addition, the emergence of Predators, Deposit Feeders, and Omnivores highlights an ecological shift toward a more balanced and trophically complex community, suggesting that changes due to IMTA installation not only mitigate organic waste impacts but also support diverse trophic interactions. The presence of alien species, although variable, did not show pronounced peaks in treatment areas compared to control sites, implying that IMTA might contribute to stability and resistance to invasion through niche occupation and competitive exclusion. However, the pronounced peak of alien species in control sites, particularly around 2020, underscores the susceptibility of monoculture systems to invasions due to altered environmental conditions, reduced diversity, and potentially increased nutrient loads.

The integration of statistical analyses provides a comprehensive understanding of the changes induced by the IMTA system on the hard-bottom macro-zoobenthic community. Multivariate analysis (PERMANOVA, PCO) confirmed significant temporal and spatial shifts in macro-zoobenthic community composition. Treatment stations exhibited more pronounced changes, indicative of a dynamic response to IMTA implementation. This is further supported by SIMPER analysis, which highlighted dominant taxa contributing to observed changes, with species like *Mytilus galloprovincialis* playing a pivotal role in structuring the community. These shifts reflect the role of environmental management in shaping benthic community dynamics. Taxonomic distinctness analysis also showed that the treatment area was characterized by slightly higher average delta+ values compared to the control area, with higher significance values observed in the summer of 2021, suggesting an improvement in taxonomic richness in the treatment area in 2021 compared to 2018, particularly given the higher reliability of the results (higher significance). In the control area, delta+ values were slightly lower and less variable compared to the treatment area, with delta+ in summer 2018 showing lower significance. In addition, lambda+, treatment stations tended to show strong differentiation, with high values and greater significance, suggesting that the taxonomic groups were well separated and distinct. In contrast, the control station showed more variable significance, suggesting that the separation may not



be as solid. Together, these analyses complement one another, offering a multi-faceted perspective on the shifts induced by the IMTA system. By connecting the results of these analyses, we demonstrate how the IMTA framework also fosters biodiversity, enhances ecological balance, and mitigates the impacts of nutrient loading on hard-bottom macro-zoobenthic communities.

By contrast, environmental parameter monitoring during this work revealed typical seasonal patterns of temperature, salinity, and dissolved oxygen in the experimental area, with an absence of dramatic fluctuations, thus suggesting that the observed community changes were more likely driven by biotic factors associated with the IMTA system rather than abiotic stressors alone.

We suggest that Mediterranean IMTA can be implemented in confined areas coupled with restoration efforts. In this way, artificial structures that mimic hard substrates can be utilized to create underwater gardens (*sensu* Rinkevich [63]) covered with typical species found within confined environments; these reef structures could be realized both on the bottom under the fish cages and within the water column as vertical gardens positioned around the cages. The presence of these vertical structures can increase both the local biodiversity and the production of the area via the elimination of waste, which can be transformed into biomass of economic value. The filtration activity carried out by Filter Feeders that settle on the vertical collectors can reduce the supply of nutrients that sink to the bottom, creating better conditions in the benthic environment. The organisms colonizing the bottom structure can also act as larval supply for vertical collectors. Moreover, the placement of artificial structures at the bottom of the water column could transform a degraded area, utilized for productive purposes, into an area in which underwater tourism can also be implemented [13].

In coastal areas, sponges, polychaetes, gorgonians, hard corals, bryozoans, bivalves, etc., contribute to realizing marine animal forests (MAFs), which represent a high percentage of biodiversity hotspots, harboring suspension feeding organisms that form erect canopies like trees or shrubs on land [64]. These marine forests immobilize blue carbon and offer diverse ecosystem services [65,66]. So-called Smart Enhanced Reefs (SERs) can be planned to increase the volume of substrates in order to facilitate the recruitment and/or transplantation of sessile organisms (algae, sponges, corals, gorgonians, etc.) and can be designed to adapt to different marine ecosystems and the specific needs of the key species (sessile or vagile) [67]. These reefs are specially designed to allow for underwater gardening for conservation and productivity purposes, as well as to increase sustainable tourism [68].

Lastly, while the findings from the present research indicate positive outcomes, further long-term monitoring is recommended to better understand the stability of these changes and the potential for IMTA systems to mitigate broader environmental impacts associated with mariculture. It is important to note that the described environmental conditions, observed from 2018 to 2021, have since undergone some changes. For instance, rising temperature and prolonged heatwaves observed since 2022 are driving shifts in the fouling community toward a different equilibrium [69], which is currently under observation.

## 5. Conclusions

Integrated Multi-Trophic Aquaculture (IMTA) systems harness interactions among species at different trophic levels to enhance water quality, reduce ecological footprints, and improve both animal welfare and product quality. While much of the existing research and monitoring has focused on soft-bottom benthic communities beneath mariculture facilities, hard-bottom macro-zoobenthic communities remain understudied despite their significant ecological roles. These communities, thriving on hard substrates within mariculture installations, play a critical role in nutrient cycling, biodiversity support, and ecosystem

stabilization. Properly designed and managed IMTA systems can transform mariculture facilities into multifunctional ecosystems. Hard-bottom macro-zoobenthic communities contribute to regenerative ocean farming by sequestering carbon, recycling nutrients, and providing essential habitats for various marine organisms. These contributions align with sustainable development goals and highlight the potential for mariculture to integrate ecological sustainability with economic productivity. Although converting traditional aquaculture facilities into IMTA systems entails increased installation and management costs, the ecological and economic benefits of fostering healthy hard-substrate benthic communities are undeniable. A polyculture approach, incorporating organisms from multiple trophic levels, can produce biomass with significant commercial value. Such an approach not only enhances profitability for aquaculture operators but also utilizes waste as an energy source, reducing the need for external feed inputs. By integrating these principles, IMTA systems can drive a circular economy in aquaculture, minimizing environmental impacts while maximizing resource efficiency. This paradigm shift offers a pathway toward a sustainable blue economy, ensuring that mariculture aligns with ecosystem health, biodiversity preservation, and long-term economic viability.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse13010143/s1>, Table S1: Presence-absence list of taxa surveyed in the treatment (A3, A6) and control (B3, B6) stations over the years (from winter 2018 to summer 2021); Table S2: Values and significance (%) of delta+ ( $\Delta+$ ) and lamda+ ( $\Lambda+$ ) across the sampling sites; Table S3: Results of SIMPER analysis showing species contributions up to 70% cumulative similarity.

**Author Contributions:** Conceptualization, C.L. and C.P.; methodology, C.L. and C.P.; validation, C.L. and C.P.; formal analysis, R.T. and A.S.; investigation, R.T., C.L., C.P., I.C., M.M. and T.L.; resources, C.L. and R.T.; data curation, R.T. and A.S.; writing—original draft preparation, C.L., A.S. and A.G.; writing—review and editing, C.L., C.P., T.L., I.C., M.M., A.S. and A.G.; visualization, R.T., A.S. and I.C.; supervision, C.L.; project administration, C.L. and R.T.; funding acquisition, C.L. and R.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by REMEDIA Life project (LIFE16 ENV/IT/000343) and by MASBIA project (Marine Sponges as Bioremediators in Integrated Aquaculture, an ERC SEEDS UNIBA cod. 2023-UNBACLE-0245506).

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** Data are contained within this article and the Supplementary Materials.

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

## References

1. Boyd, C.E.; McNevin, A.A.; Davis, R.P. The Contribution of Fisheries and Aquaculture to the Global Protein Supply. *Food Secur.* **2022**, *14*, 805–827. [[CrossRef](#)] [[PubMed](#)]
2. Costello, C.; Cao, L.; Gelcich, S.; Cisneros-Mata, M.Á.; Free, C.M.; Froehlich, H.E.; Golden, C.D.; Ishimura, G.; Maier, J.; Macadam-Somer, I.; et al. The Future of Food from the Sea. *Nature* **2020**, *588*, 95–100. [[CrossRef](#)] [[PubMed](#)]
3. Karakassis, I.; Tsapakis, M.; Hatziyanni, E.; Papadopoulou, K.-N.; Plaiti, W. Impact of Cage Farming of Fish on the Seabed in Three Mediterranean Coastal Areas. *ICES J. Mar. Sci.* **2000**, *57*, 1462–1471. [[CrossRef](#)]
4. Maldonado, M.; Carmona, M.C.; Echeverría, Y.; Riesgo, A. The Environmental Impact of Mediterranean Cage Fish Farms at Semi-Exposed Locations: Does It Need a Re-Assessment? *Helgol. Mar. Res.* **2005**, *59*, 121–135. [[CrossRef](#)]
5. De Silva, S.S. Aquaculture: A Newly Emergent Food Production Sector—And Perspectives of Its Impacts on Biodiversity and Conservation. *Biodivers. Conserv.* **2012**, *21*, 3187–3220. [[CrossRef](#)]
6. Dunlop, K.; Harendza, A.; Bannister, R.; Keeley, N. Spatial Response of Hard- and Mixed-Bottom Benthic Epifauna to Organic Enrichment from Salmon Aquaculture in Northern Norway. *Aquac. Environ. Interact.* **2021**, *13*, 455–475. [[CrossRef](#)]
7. Pascal, L.; Chaillou, G.; Nozais, C.; Cool, J.; Bernatchez, P.; Letourneux, K.; Archambault, P. Benthos Response to Nutrient Enrichment and Functional Consequences in Coastal Ecosystems. *Mar. Environ. Res.* **2022**, *175*, 105584. [[CrossRef](#)]

8. Borghese, J.; Giangrande, A.; Arduini, D.; Trani, R.; Doria, L.; Anglano, M.; Rizzo, L.; Rossi, S. Influence of an Innovative IMTA System (Mediterranean Sea, Italy) on Environmental and Biological Parameters: Seasonal Analysis. *Aquaculture* **2025**, *596*, 741726. [[CrossRef](#)]
9. Rousi, H.; Laine, A.O.; Peltonen, H.; Kangas, P.; Andersin, A.-B.; Rissanen, J.; Sandberg-Kilpi, E.; Bonsdorff, E. Long-Term Changes in Coastal Zoobenthos in the Northern Baltic Sea: The Role of Abiotic Environmental Factors. *ICES J. Mar. Sci.* **2013**, *70*, 440–451. [[CrossRef](#)]
10. Alleway, H.K.; Gillies, C.L.; Bishop, M.J.; Gentry, R.R.; Theuerkauf, S.J.; Jones, R. The Ecosystem Services of Marine Aquaculture: Valuing Benefits to People and Nature. *BioScience* **2019**, *69*, 59–68. [[CrossRef](#)]
11. Nissar, S.; Bakhtiyar, Y.; Arafat, M.Y.; Andrabi, S.; Mir, Z.A.; Khan, N.A.; Langer, S. The Evolution of Integrated Multi-Trophic Aquaculture in Context of Its Design and Components Paving Way to Valorization via Optimization and Diversification. *Aquaculture* **2023**, *565*, 739074. [[CrossRef](#)]
12. Arduini, D.; Borghese, J.; Gravina, M.F.; Trani, R.; Longo, C.; Pierri, C.; Giangrande, A. Biofouling Role in Mariculture Environment Restoration: An Example in the Mar Grande of Taranto (Mediterranean Sea). *Front. Mar. Sci.* **2022**, *9*, 842616. [[CrossRef](#)]
13. Giangrande, A.; Gravina, M.F.; Rossi, S.; Longo, C.; Pierri, C. Aquaculture and Restoration: Perspectives from Mediterranean Sea Experiences. *Water* **2021**, *13*, 991. [[CrossRef](#)]
14. Giangrande, A.; Licciano, M.; Arduini, D.; Borghese, J.; Pierri, C.; Trani, R.; Longo, C.; Petrocelli, A.; Ricci, P.; Alabiso, G.; et al. An Integrated Monitoring Approach to the Evaluation of the Environmental Impact of an Inshore Mariculture Plant (Mar Grande of Taranto, Ionian Sea). *Biology* **2022**, *11*, 617. [[CrossRef](#)] [[PubMed](#)]
15. Bevilacqua, S.; Katsanevakis, S.; Micheli, F.; Sala, E.; Rilov, G.; Sarà, G.; Malak, D.A.; Abdulla, A.; Gerovasileiou, V.; Gissi, E.; et al. The Status of Coastal Benthic Ecosystems in the Mediterranean Sea: Evidence From Ecological Indicators. *Front. Mar. Sci.* **2020**, *7*, 475. [[CrossRef](#)]
16. Dauvin, J.-C.; Fersi, A.; Pezy, J.-P.; Bakalem, A.; Neifar, L. Macrobenthic Communities in the Tidal Channels around the Gulf of Gabès, Tunisia. *Mar. Pollut. Bull.* **2021**, *162*, 111846. [[CrossRef](#)]
17. Giangrande, A.; Licciano, M.; Musco, L. Polychaetes as Environmental Indicators Revisited. *Mar. Pollut. Bull.* **2005**, *50*, 1153–1162. [[CrossRef](#)]
18. Buschmann, A.H.; Riquelme, V.A.; Hernández-González, M.C.; Varela, D.; Jiménez, J.E.; Henríquez, L.A.; Vergara, P.A.; Guíñez, R.; Filún, L. A Review of the Impacts of Salmonid Farming on Marine Coastal Ecosystems in the Southeast Pacific. *ICES J. Mar. Sci.* **2006**, *63*, 1338–1345. [[CrossRef](#)]
19. Forchino, A.; Borja, A.; Brambilla, F.; Rodríguez, J.G.; Muxika, I.; Terova, G.; Saroglia, M. Evaluating the Influence of Off-Shore Cage Aquaculture on the Benthic Ecosystem in Alghero Bay (Sardinia, Italy) Using AMBI and M-AMBI. *Ecol. Indic.* **2011**, *11*, 1112–1122. [[CrossRef](#)]
20. Kalantzi, I.; Karakassis, I. Benthic Impacts of Fish Farming: Meta-Analysis of Community and Geochemical Data. *Mar. Pollut. Bull.* **2006**, *52*, 484–493. [[CrossRef](#)]
21. Moraitis, M.; Papageorgiou, N.; Dimitriou, P.D.; Petrou, A.; Karakassis, I. Effects of Offshore Tuna Farming on Benthic Assemblages in the Eastern Mediterranean. *Aquac. Environ. Interact.* **2013**, *4*, 41–51. [[CrossRef](#)]
22. Tanner, J.E.; Williams, K. The Influence of Finfish Aquaculture on Benthic Fish and Crustacean Assemblages in Fitzgerald Bay, South Australia. *PeerJ* **2015**, *3*, e1238. [[CrossRef](#)] [[PubMed](#)]
23. Karakassis, I.; Hatziyanni, E.; Tsapakis, M.; Plaiti, W. Benthic Recovery Following Cessation of Fish Farming: A Series of Successes and Catastrophes. *Mar. Ecol. Prog. Ser.* **1999**, *184*, 205–218. [[CrossRef](#)]
24. Huguenin, L.; Lalanne, Y.; Bru, N.; Lissardy, M.; D'Amico, F.; Monperrus, M.; de Casamajor, M.-N. Identifying Benthic Macrofaunal Assemblages and Indicator Taxa of Intertidal Boulder Fields in the South of the Bay of Biscay (Northern Basque Coast). A Framework for Future Monitoring. *Reg. Stud. Mar. Sci.* **2018**, *20*, 13–22. [[CrossRef](#)]
25. Borghese, J.; Musco, L.; Arduini, D.; Tamburello, L.; Del Pasqua, M.; Giangrande, A. A Comparative Approach to Detect Macrobenthic Response to the Conversion of an Inshore Mariculture Plant into an IMTA System in the Mar Grande of Taranto (Mediterranean Sea, Italy). *Water* **2023**, *15*, 68. [[CrossRef](#)]
26. Stabili, L.; Giangrande, A.; Arduini, D.; Borghese, J.; Petrocelli, A.; Alabiso, G.; Ricci, P.; Cavallo, R.A.; Acquaviva, M.I.; Narracci, M.; et al. Environmental Quality Improvement of a Mariculture Plant after Its Conversion into a Multi-Trophic System. *Sci. Total Environ.* **2023**, *884*, 163846. [[CrossRef](#)]
27. Carballeira Braña, C.B.; Cerbule, K.; Senff, P.; Stolz, I.K. Towards Environmental Sustainability in Marine Finfish Aquaculture. *Front. Mar. Sci.* **2021**, *8*, 666662. [[CrossRef](#)]
28. Keeley, N.; Laroche, O.; Birch, M.; Pochon, X. A Substrate-Independent Benthic Sampler (SIBS) for Hard and Mixed-Bottom Marine Habitats: A Proof-of-Concept Study. *Front. Mar. Sci.* **2021**, *8*, 627687. [[CrossRef](#)]
29. Mahmood, T.; Fang, J.; Jiang, Z.; Zhang, J. Carbon and Nitrogen Flow, and Trophic Relationships, among the Cultured Species in an Integrated Multi-trophic Aquaculture (IMTA) Bay. *Aquac. Environ. Interact.* **2016**, *8*, 207–219. [[CrossRef](#)]

30. Ning, Z.; Liu, S.; Zhang, G.; Ning, X.; Li, R.; Jiang, Z.; Fang, J.; Zhang, J. Impacts of an Integrated Multi-Trophic Aquaculture System on Benthic Nutrient Fluxes: A Case Study in Sanggou Bay, China. *Aquac. Environ. Interact.* **2016**, *8*, 221–232. [CrossRef]
31. Chopin, T.; Cooper, J.A.; Reid, G.; Cross, S.; Moore, C. Open-Water Integrated Multi-Trophic Aquaculture: Environmental Bioremediation and Economic Diversification of Fed Aquaculture by Extractive Aquaculture. *Rev. Aquac.* **2012**, *4*, 209–220. [CrossRef]
32. Hossain, A.; Senff, P.; Glaser, M. Lessons for Coastal Applications of IMTA as a Way towards Sustainable Development: A Review. *Appl. Sci.* **2022**, *12*, 11920. [CrossRef]
33. Giangrande, A.; Pierri, C.; Arduini, D.; Borghese, J.; Licciano, M.; Trani, R.; Corriero, G.; Basile, G.; Cecere, E.; Petrocelli, A.; et al. An Innovative IMTA System: Polychaetes, Sponges and Macroalgae Co-Cultured in a Southern Italian In-Shore Mariculture Plant (Ionian Sea). *J. Mar. Sci. Eng.* **2020**, *8*, 733. [CrossRef]
34. Aguilo-Arce, J.; Ferriol, P.; Puthod, P.; Trani, R.; Longo, C. The Remedial Life Integrated Multitrophic Aquaculture System as a Powerful Sponge Biomass Supply. *Biol. Mar. Mediterr.* **2024**, *28*, 87–89.
35. Trani, R.; Aguilo-Arce, J.; Ferriol, P.; Puthod, P.; Pierri, C.; Longo, C. Metodologie di Allevamento di Poriferi in un Sistema di Acquacoltura Multi Trofica Integrata. *Biol. Mar. Mediterr.* **2024**, *28*, 94–97.
36. Occhipinti-Ambrogi, A. Biotic Invasions in a Mediterranean Lagoon. *Biol. Invasions* **2000**, *2*, 165–176. [CrossRef]
37. Marchini, A.; Gauzer, K.; Occhipinti-Ambrogi, A. Spatial and Temporal Variability of Hard-Bottom Macrofauna in a Disturbed Coastal Lagoon (Sacca Di Goro, Po River Delta, Northwestern Adriatic Sea). *Mar. Pollut. Bull.* **2004**, *48*, 1084–1095. [CrossRef]
38. WoRMS—World Register of Marine Species. Available online: <https://www.marinespecies.org/wormsliterature.php> (accessed on 9 December 2024).
39. Eaton, L. Development and Validation of Biocriteria Using Benthic Macroinvertebrates for North Carolina Estuarine Waters. *Mar. Pollut. Bull.* **2001**, *42*, 23–30. [CrossRef]
40. Sconfietti, R.; Marino, R. Patterns of Zonation of Sessile Macrobenthos in a Lagoon Estuary (Northern Adriatic Sea). *Sci. Mar.* **1989**, *53*, 145–154.
41. Garrison, J.A.; Nordström, M.C.; Albertsson, J.; Nascimento, F.J.A. Temporal and Spatial Changes in Benthic Invertebrate Trophic Networks along a Taxonomic Richness Gradient. *Ecol. Evol.* **2022**, *12*, e8975. [CrossRef]
42. Bullivant, J.S. The Method of Feeding of Lophophorates (Bryozoa, Phoronida, Brachiopoda). *N. Z. J. Mar. Freshw. Res.* **1968**, *2*, 135–146. [CrossRef]
43. Fauchald, K.; Jumars, P. The Diet of Worms: A Study of Polychaete Feeding Guilds. *Ocean. Mar. Biol.* **1979**, *17*, 193–284.
44. Leonard, A.B. Functional Response in *Antedon mediterranea* (Lamarck) (Echinodermata: Crinoidea): The Interaction of Prey Concentration and Current Velocity on a Passive Suspension-Feeder. *J. Exp. Mar. Biol. Ecol.* **1989**, *127*, 81–103. [CrossRef]
45. Calvo, M.; Templado, J. Reproduction and Development in a Vermetid Gastropod. *Vermetus Triquetrus. Invertebr. Biol.* **2004**, *123*, 289–303. [CrossRef]
46. Riisgård, H.U.; Larsen, P.S. Water Flow Analysis and Particle Capture in Ciliary Suspension-Feeding Scallops (Pectinidae). *Mar. Ecol. Prog. Ser.* **2005**, *303*, 177–193. [CrossRef]
47. Gaudêncio, M.J.; Cabral, H.N. Trophic Structure of Macrobenthos in the Tagus Estuary and Adjacent Coastal Shelf. *Hydrobiologia* **2007**, *587*, 241–251. [CrossRef]
48. Morton, B.; Peharda, M.; Harper, E.M. Drilling and Chipping Patterns of Bivalve Prey Predation by *Hexaplex trunculus* (Mollusca: Gastropoda: Muricidae). *J. Mar. Biol. Assoc. U. K.* **2007**, *87*, 933–940. [CrossRef]
49. Petersen, J.K. Ascidian Suspension Feeding. *J. Exp. Mar. Biol. Ecol.* **2007**, *342*, 127–137. [CrossRef]
50. Schlesinger, A.; Zlotkin, E.; Kramarsky-Winter, E.; Loya, Y. Cnidarian Internal Stinging Mechanism. *Proc. R. Soc. B Biol. Sci.* **2008**, *276*, 1063–1067. [CrossRef]
51. Tlig-Zouari, S.; Rabaoui, L.; Irathni, I.; Ben Hassine, O.K. Distribution, Habitat and Populations Densities of the Invasive Species *Pinctada Radiata* (Mollusca: Bivalvia) along the Northern and Eastern Coasts of Tunisia. *Cah. Biol. Mar.* **2009**, *50*, 131–142.
52. Zubikarai, M.; Borja, A.; Muxika, I. Assessment of Benthic Hard Substratum Communities Responses to Changes in the Management of Anthropogenic Pressures in the Basque Coast. *Rev. Investig. Mar.* **2014**, *21*, 40–88.
53. Jumars, P.A.; Dorgan, K.M.; Lindsay, S.M. Diet of Worms Emended: An Update of Polychaete Feeding Guilds. *Annu. Rev. Mar. Sci.* **2015**, *7*, 497–520. [CrossRef] [PubMed]
54. Riisgård, H.U. Filter-Feeding Mechanisms in Crustaceans. In *Lifestyles and Feeding Biology*; Oxford University Press: Oxford, UK, 2015; pp. 418–463, ISBN 978-0-19-979702-8.
55. Shannon, C.E.; Weaver, W. *The Mathematical Theory of Communication*; University of Illinois Press: Urbana, IL, USA, 1963; pp. 210–360.
56. Pielou, E.C. *An Introduction to Mathematical Ecology*; Wiley: New York, NY, USA, 1969.
57. Margalef, R. Information theory in ecology. *Gen. Syst.* **1958**, *3*, 36–71.
58. Clarke, K.R.; Warwick, R.M. A Further Biodiversity Index Applicable to Species Lists: Variation in Taxonomic Distinctness. *Mar. Ecol. Prog. Ser.* **2001**, *216*, 265–278. [CrossRef]



59. Clarke, K.R.; Warwick, R.M. Similarity-Based Testing for Community Pattern: The Two-Way Layout with No Replication. *Mar. Biol.* **1994**, *118*, 167–176. [[CrossRef](#)]
60. Anderson, M.J. Permutational Multivariate Analysis of Variance. *Dep. Stat. Univ. Auckl. Auckl.* **2005**, *26*, 32–46.
61. Clarke, K.R. Non-Parametric Multivariate Analyses of Changes in Community Structure. *Aust. J. Ecol.* **1993**, *18*, 117–143. [[CrossRef](#)]
62. Anderson, M. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; Primer-E Ltd.: Plymouth, UK, 2008.
63. Rinkevich, B. Ecological Engineering Approaches in Coral Reef Restoration. *ICES J. Mar. Sci.* **2021**, *78*, 410–420. [[CrossRef](#)]
64. Rossi, S. The Destruction of the ‘Animal Forests’ in the Oceans: Towards an over-Simplification of the Benthic Ecosystems. *Ocean Coast. Manag.* **2013**, *84*, 77–85. [[CrossRef](#)]
65. Rossi, S.; Bramanti, L.; Gori, A.; Orejas, C. Animal Forests of the World: An Overview. In *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*; Rossi, S., Bramanti, L., Gori, A., Orejas, C., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 1–28. [[CrossRef](#)]
66. Paoli, C.; Montefalcone, M.; Morri, C.; Vassallo, P.; Bianchi, C.N. Ecosystem functions and services of the marine animal forests. In *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*; Springer: Cham, Switzerland, 2017; ISBN 9783319210124.
67. Rossi, S.; Rizzo, L. Marine animal forests as carbon immobilizers or why we should preserve these three-dimensional alive structures. In *Perspectives on the Marine Animal Forests of the World*; Springer: Cham, Switzerland, 2020.
68. Westoby, R.; Becken, S.; Laria, A.P. Perspectives on the Human Dimensions of Coral Restoration. *Reg. Environ. Change* **2020**, *20*, 109. [[CrossRef](#)]
69. Arduini, D.; Portacci, G.; Giangrande, A.; Acquaviva, M.I.; Borghese, J.; Calabrese, C.; Giandomenico, S.; Quarta, E.; Stabili, L. Growth Performance of *Mytilus Galloprovincialis* Lamarck, 1819 under an Innovative Integrated Multi-Trophic Aquaculture System (IMTA) in the Mar Grande of Taranto (Mediterranean Sea, Italy). *Water* **2023**, *15*, 1922. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.