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Environmental quality improvement of a mariculture plant after its conversion into a multi-trophic system

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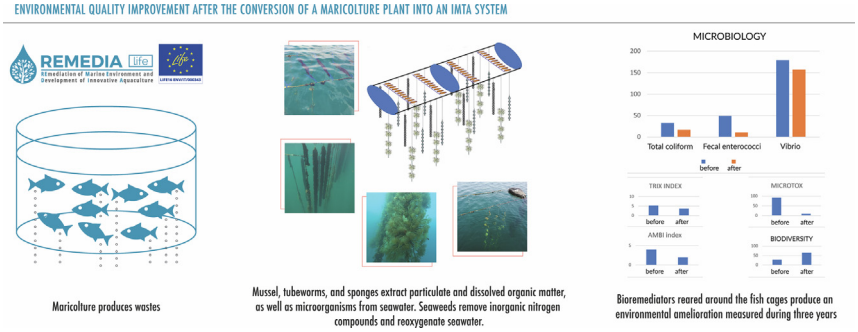
HIGHLIGHTS

- Fish farming can have a strong impact on seawater eutrophication in coastal waters.
- Many aquaculture species efficiently bioremediate inorganic and/or organic nutrients.
- Integrated Multitrophic Aquaculture allows the polyculture of several bioremediators.
- Mussels, sponges, tubeworms, and seaweeds were farmed successfully in a fish farm.
- IMTA proved to be the new frontier for sustainable aquaculture and circular economy.

GRAPHICAL ABSTRACT



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ABSTRACT

Integrated Multitrophic Aquaculture (IMTA) seems to be one of the best solutions for sustainable aquaculture. Within the Remedia LIFE Project, an experimental IMTA plant was put in place in the Mar Grande of Taranto (Mediterranean Sea, Southern Italy). The polyculture of several bioremediating organisms, such as mussels, tubeworms, sponges, and seaweeds, was combined with a coastal cage fish farm, in order to remove organic and inorganic wastes coming from the fish's metabolism. To verify the effectiveness of the system, the *ex ante* measurement of chemical-physical variables, trophic status, microbial contamination, and zoobenthos community health was compared with the results of the same measurement performed one year and two years after the implementation of the experimental IMTA plant. The results were encouraging, since a reduction in total nitrogen concentration in the seawater (from 43.4 ± 8.9 to 5.6 ± 3.7 μM/l), a reduction in microbial pollution indicators in the seawater (total coliforms: from 280 ± 18 MPN/100 mL to 0; *E. coli*: from 33 ± 1.3 MPN/100 mL to 0) and in the sediments (total coliforms: from 230 ± 6.2 MPN/100 g to 170 ± 9; *E. coli*: from 40 ± 9.4 MPN/100 g to 0), an enhancement of the trophic status (TRIX: from 4.45 ± 1.29 to 3.84 ± 0.18), and an increase in the zoobenthic quality indices and biodiversity were recorded (AMBI: from 4.8 to 2.4; M-AMBI: from 0.14 to 0.7). These results prove that the Remedia LIFE project's purpose was achieved. The selected

Abbreviations: IMTA, Integrated Multitrophic Aquaculture.

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bioremediators worked synergistically, improving water and sediments quality within the fish farm area. Moreover, bioremediating organisms increased their weight as a result of wastes uptake, producing, as co-products, large amounts of additional biomass. This could be commercially exploited, thus being an added value of the IMTA plant. Based on our findings, the promotion of eco-friendly practices to ameliorate ecosystem health should be encouraged.

1. Introduction

The rapid world population growth is generating a greater demand for food, leading to the overexploitation of resources - particularly for animal consumption - impacting species worldwide, and causing environmental degradation (Ripple et al., 2020). Aquaculture, especially mariculture, represents the fastest-growing form of food production (FAO, 2022). It is one of the most sustainable ways to produce animal proteins and it is one of the EU's Blue Growth Strategy's sectors for boosting economic growth in Europe (Duarte et al., 2009; Alleway et al., 2018; Brugere et al., 2018; Custódio et al., 2019). One-third of the world's seafood resources comes from the mariculture industry and sustainable aquaculture production is highly important to face future protein demands (Jones et al., 2022). However, mariculture still has several problematic aspects, such as its environmental impact, fodder production, as well as issues linked to the types of farmed species and the choice of suitable sites (Serpa and Duarte, 2008; Costello et al., 2020). In coastal zones with high anthropogenic pressure, a strong interest towards offshore aquaculture is growing in order to limit the impact of eutrophication and overcome shortage of space (IUCN, 2009a, 2009b; Mizuta et al., 2019). However, around the world, inshore mariculture production is carried out in shallow waters with low hydrodynamic energy and close to mainland supporting infrastructures (Osmundsen et al., 2020) and these plants strongly affect their surrounding environment (Muir et al., 1999; Gentry et al., 2017). Wastes deriving from mariculture activities often have a negative environmental impact, affecting water quality and benthic communities (Wang et al., 2020). Moreover, uneaten feed leads to the deterioration of water quality and disease outbreaks (Stabili et al., 2010), contributing to the growing concern for the rate of diseases in the aquaculture industry worldwide. For this reason, higher concentrations of antibiotics are given to fish, therefore aquaculture has regularly been blamed for being unsustainable and non-environmentally friendly (González-Gaya et al., 2022; Wang et al., 2022).

Research on the bioremediation of aquaculture has gained momentum over the last two decades and now the time is ripe to improve the related technologies and reduce the environmental footprint of this economic activity. One of the best strategies to overcome the impact of mariculture inshore plants could be Integrated Multitrophic Aquaculture (IMTA) (Buck et al., 2018). In this system, the polyculture of different organisms belonging to different trophic levels can bioremediate the increased nutrient load in the water column and sediments (Fang et al., 2016; Li et al., 2021; Naskar et al., 2022). This farming technique has other benefits too, such as the possibility to diversify production by obtaining additional commercially exploitable biomass (Knowler et al., 2020; Thomas et al., 2021).

However, although the detrimental effects of fish farming have been assessed in many studies (Karakassis et al., 2000; Mazzola et al., 2000; Vezzulli et al., 2002), to date there are very few researches estimating in situ positive effect of IMTA systems, and none of them was performed in the Mediterranean area (Cheshuk et al., 2003; Fang et al., 2016; Mahmood et al., 2016; Ning et al., 2016; Nederlof et al., 2020; Li et al., 2021; Naskar et al., 2022). Indeed, although IMTA systems are famous for reducing the environmental impact of aquaculture, so far, their beneficial effects on seawater quality have been mostly inferred from the innate bioremediation capabilities of the selected co-cultured organisms, rather than quantitatively assessed through in situ seawater quality assessments. In this respect, project Remedia LIFE (LIFE16 ENV/IT/000343) is being carried out in the Mar Grande of Taranto (Southern Italy, Ionian Sea, Mediterranean Sea) mainly aiming to experiment with commercially exploitable organisms such as sponges, tubeworms, mussels, and seaweeds as bioremediating organisms. To this end, part of a fish mariculture plant was converted into an innovative IMTA system that proved to be efficient in terms of production (Giangrande et al., 2020). To identify the most suitable site for the placement of the experimental modules, an accurate monitoring survey of seawater, sediments, and zoobenthos was performed at four sampling stations at the beginning of the project (Giangrande et al., 2022). To test the hypothesis that the presence of bioremediators could have a possible restorative effect on the surrounding environment, this monitoring program was then repeated one and two years after the IMTA system implementation. Here we report the comparison of the environmental and biological variables measured at different times.

2. Materials and methods

2.1. Study area

The study was carried out in the fishing farm “Maricoltura Mar Grande”, located in the Mar Grande basin (40°25′56″ N; 17°14′19″ E) (Fig. 1) approximately 600 m far from the coast, and having a surface area of 0.06 km^2. The Mar Grande of Taranto is a semi-enclosed basin connected to the Gulf of Taranto. Temperatures show seasonal variations that are typical of the coastal Ionian regions, with an average annual value of about 18 °C, while salinity, almost uniform over the year, is about 38 psu. Intense anthropogenic activities affect the area, including mussel farming facilities.

The fish farm plant consists of 15 cages (Ø 22 m) arranged in three rows of five cages each and placed at a depth ranging from −7 to −12 m, where European seabass *Dicentrarchus labrax* (Linnaeus, 1758) and sea bream *Sparus aurata* (Linnaeus, 1758) are farmed with a production level of about 100 tons/year.

The *ex ante* analysis revealed that an area of the plant was more impacted by eutrophication (Giangrande et al., 2022) and it was therefore chosen for the bioremediation system. Three long-line structures (LLA, LLB, LLC), each consisting of a double series of 10 floats held together with ropes, were placed around six cages (Fig. 1) and each of them was anchored to two buoys to prevent it from sinking. In different seasons, bioremediating organisms, that is the *Sabella spallanzanii* (Gmelin, 1791) tube worm, the *Mytilus galloprovincialis* (Lamarck, 1819) mussel, the *Sarcotragus spinosulus* (Schmidt, 1862) sponge, and the *Chaetomorpha linum* (O.F. Müller) Kützing and *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C Silva seaweeds were hung on the long-lines. Tube worms were laid on 184 coconut fiber ropes, while sponges, mussels, and seaweeds were placed in tubular plastic nets commonly used for mussel farming. The sponge-rearing modules consisted of 7 m long ropes in which sponge explants, wrapped in plastic nets, were inserted every 40 cm, for a total of 12–15 explants per rope, while the macroalgae, once collected in the natural environment, were transferred to the aquaculture farm to set up the cultivation sockets, each consisting of seaweeds enclosed into a net sack and hung with a festoon arrangement at a depth of about 1 m. The two species of seaweed, *C. linum* and *G. bursa-pastoris,* were tested in two different seasons because they have different life cycles. A total of 186 cultivation sockets (99 for the *C. linum* and 87 for the *G. bursa-pastoris*) were placed in the plant. A detailed description of the experimental IMTA system as well as the growth performances of reared organisms, and the obtained biomass (Supplementary Table 1) is reported by Stabili et al. (2019) and Giangrande et al. (2020). In this area, Station A was chosen for the next yearly monitorings. Another station, Station B, located in the opposite area without long-lines, was chosen as the Control (Fig. 1).

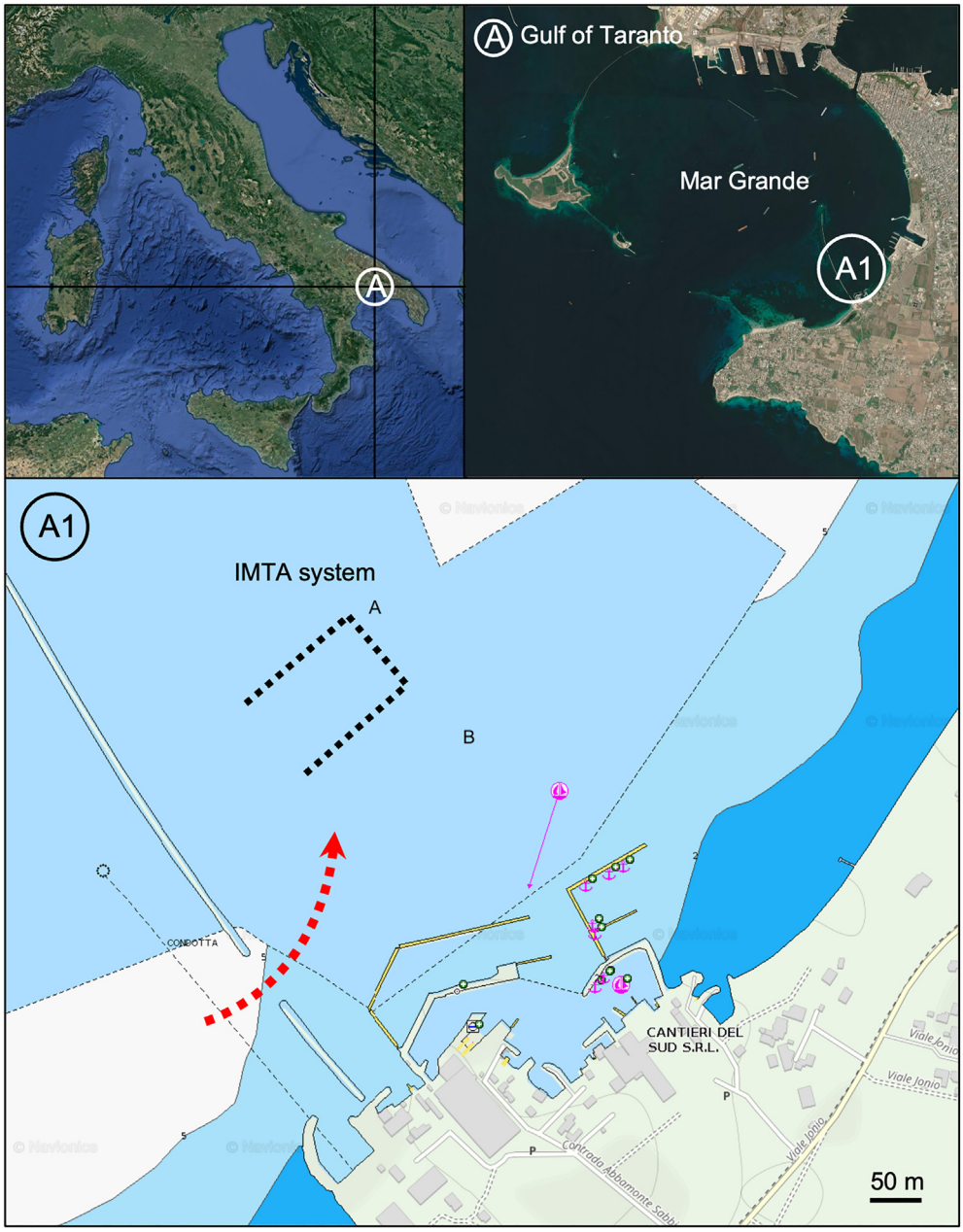

Fig. 1. Map of the study site. Dashed line indicate the long lines (LLA, LLB, LLC) placed in the treatment area. A: treatment sampling station; B: control sampling station. In the frame top right, the localization of Taranto in Italy.

2.2. Sampling procedures

Seawater samples for nutrient and microbiological analysis were collected in triplicate at the surface (1 m depth) using a Niskin bottle (ABT-NK-02, Aquatic BioTechnology, Cádiz, Spain) throughout the experimental period (July 2018, 2019, and 2020). Sediment samples for the microbiological investigations were collected in triplicate from under the cages at a 12 m depth at each site by scuba divers and preserved under sterile conditions.

The seabed under the cages was mainly composed of mud. For each sampling site three replicates of soft bottom were collected for macrobenthic index analysis using a 15×10^3 mm^3 grab sampler (Ekman Standard, Scubla, Remanzacco, Italy). Sediment samples were sieved on site through a 0.5 mm mesh and the individuals retained were preserved in 70 % ethanol solution.

The hard bottom macrobenthic community growing on artificial hard substrates (i.e., concrete anchoring blocks and chains for fish cage anchoring) was also investigated. At each station, photographs were taken and subsequently analyzed at a lab with the ImageJ software, annotating the conspicuous fauna species and determining their surface coverage. Samples for macrobenthic index measurements were also collected by scraping off three replicates of 4×10^{-2} m^2.

2.3. Chemical-physical measurements

Temperature (°C), salinity, and pH were measured in triplicate on the surface at each site using a multiparametric probe (IDROMAR, IP050D, San Giuliano Milanese, Italy).

2.4. Nutrient analysis and trophic status

Water samples were analyzed to assess the nutrient content using a multiparameter laboratory analyzer (Systea Srl Micromac Lab 1000, Anagni, Italy) and following the APAT and IRSA-CNR (2003a) methods. Concentrations of N-NH_3, N-NO_3, N-NO_2, P-PO_4 and total N and P were measured. For Chlorophyll *a*, seawater was filtered in acetone 90 % on 47

Ø mm Whatman GF/F filters and then measured fluorometrically. These measures were propaedeutic to the assessment of the trophic conditions of the seawater, which was performed calculating the TRIX index (Pettine et al., 2007):

$$TRIX = [\log 10\,(Chl\,a \times D\%O_2 \times DIN \times P) - (-1.5)]/1.2$$

where:

Chl a = chlorophyll a ($\mu g\,l^{-1}$);

D% O_2 = % deviation of the oxygen concentration from saturation conditions;

DIN = dissolved inorganic nitrogen (μM/l);

P = total phosphorus (μM/l).

Each interval of TRIX values corresponds to an environmental "Quality Rating" ranging from High to Poor ($2 < TRIX < 4$ = High; $4 \leq TRIX < 5$ = Good; $5 \leq TRIX < 6$ = Moderate; $6 \leq TRIX < 8$ = Poor) (Pettine et al., 2007).

2.5. Microbiology

Samples from both compartments (i.e., water and sediments) were transported to the microbiology laboratory within 4 h after sampling and then used in the microbiological analyses. In particular, the following parameters were investigated: culturable vibrios, total coliforms, fecal coliforms, fecal enterococci, *Escherichia coli*, and *Salmonella*. Sediment samples were diluted with filtered (0.22 μm) seawater to obtain a 1:10 (*w/v*) dilution and homogenized for 90 s in a sterile Waring blender. The homogenates were then processed in a similar manner to the seawater samples. In order to assess the microbial water quality, standard methods (e.g. ISO, the International Organization for Standardization) were chosen. For the enumeration of culturable vibrios, 1, 5, and 10 mL of each sample (seawater and sediment) were filtered in triplicate on 0.45 μm Millipore pore size filters (Merck KGaA, Darmstadt, Germany) that were aseptically placed onto thiosulphate-citrate-bile-salt-agar (TCBS) plus 2 % NaCl. After incubation for 48 h at 22 and 35 °C, the culturable vibrios developed were counted and presented in terms of the colony-forming unit (CFU) (Stabili et al., 2006a). The incubation temperature of 35 °C was chosen to estimate the fraction of vibrios potentially pathogenic to humans. The lowest incubation temperature (20–25 °C) was selected to detect some *Vibrio* spp., including *V. anguillarum*, that do not thrive at 37 °C (Planas et al., 2006). After incubation, the colonies of presumptive vibrios (yellow or green), grown on TCBS agar, were counted according to the colony-forming unit (CFU) method. Total coliforms, fecal coliforms, and fecal enterococci were evaluated by using the most probable number (MPN) method, and the standard five-tube method of ten-fold dilutions for seawater samples (APHA, 2005). The coliform bacteria concentration was determined by using the miniaturized MPN, in accordance with ISO 9308-3:1998 (ISO, 1998a). Fecal enterococci were counted by using the miniaturized MPN method (incubation at 44 °C for 24–48 h) (ISO, 1998b). Results were referred as MPN 100 ml^{-1} or 100 g^{-1} for water and sediment samples respectively. The enumeration of *Escherichia coli* was carried out with a five-tube MPN method at three dilutions according to the APAT CNR IRSA 7030 procedures (APAT and IRSA-CNR, 2003b). To count *Salmonella* bacteria the UNI EN ISO 6579:2008 method was used for sediment samples and the APAT CNR IRSA 7080 procedure was used for seawater samples (APAT and IRSA-CNR, 2003c; ISO, 2008). Moreover, the acute toxicity assessment of the selected sites was performed using the Microtox assay and the *Vibrio fischeri* bacteria. Bacteria were obtained from AZUR Environmental (Carlsbad, CA, USA) as freeze-lyophilised cells. Specifically, the Microtox® Solid Phase Test (SPT) was performed on sediments: the first step was the centrifugation of samples at 8000 rpm for 30 min to remove the interstitial water, then a subsample of 7 g (±0.01 g) was used to prepare a suspension with 35 mL of diluent (Microtox® Solid Phase Test Diluent). *Vibrio fischeri* tests were exposed to a series of 1:2 dilutions of the suspension and their light emission was determined after incubation for 20 min; then, the samples were filtered using the specific tube filter systems supplied by AZUR Environmental. The light emission of the bacteria in the seawater was measured after 5 and 15 min and compared to an aqueous control. The tests were performed at 15 °C and pH 8.0 ± 0.5, with two replicates and four controls, according to the standard operating procedure. At the end of the test, each turbidity dilution was corrected using UV spectrophotometry (Lambda 3B spectrophotometer, Perkin Elmer, Waltham, MA, USA) at 490 nm. The Microtox® Basic Test (BT) was used for interstitial water (Microbics Corporation, 1994): samples were diluted 1:10 using the diluent reagent (Microtox® Diluent, Modern Water, London, UK) and the light emission of the bacteria was compared to an aqueous control. The tests were performed at 15 °C and pH 8.0 ± 0.5 with the control (Narracci et al., 2014). The toxicity assessment of the water samples was presented as the percentage of inhibition of bioluminescence of *V. fischeri*, while the test result for the sediments was presented as its toxicity index (STI) (Onorati et al., 1999).

2.6. Benthic communities

Benthic samples were washed in the laboratory and stored in 70 % ethanol solution. After sorting, specimens were identified to the highest possible taxonomic level and counted. To define the status of environmental quality, species richness was considered for hard substrates (Arduini et al., 2022), while for soft bottoms, AMBI and M-AMBI indices were calculated (Muxika et al., 2007; Borja et al., 2009). The AMBI method considers five ecological groups (EG), from EG I, including the most disturbance-sensitive species, to EG V, the first-order opportunistic species (Borja et al., 2000). The index was calculated with the following formula:

$$AMBI = \frac{[(0 \times \%EGI) + (1.5 \times \%EGII) + (3 \times \%EGIII) + (4.5 \times \%EGIV) + (6 \times \%EGV)]}{100.}$$

M-AMBI integrates the AMBI biotic index, the Shannon-Wiener diversity index (H′), and the number of species (S). M-AMBI values were obtained using the AMBI Software (version 6.0) (http://ambi.azti.es) and the updated May 2022 species list.

The values of these biotic indices reflect the quality of the marine environment and are widely used in the context of the Water Framework Directive, as well as the Marine Strategy Framework Directive, to detect different impact sources (Muxika et al., 2007). The AMBI index varies from the highest score 7 in disturbed areas to the lowest score 0 in pristine ones. Conversely, M-AMBI varies from 0 in disturbed areas to 1 in pristine ones.

2.7. Data analysis

Non-metric multidimensional scaling (nMDS), performed on the average values of the measured nutrients, was used to visualize similarities across sampling times (July 2018, July 2019, and July 2020) and stations (A and B). Data were firstly ln (x + 1) transformed and a triangular similarity matrix was obtained applying the Bray-Curtis index. Measured variables were added as overlay vectors on the nMDS plot.

A two-way analysis of variance (ANOVA) was run on microbiological parameters to test for differences among stations and sampling times. Significance was set at a critical level of 95 % ($p < 0.05$). When significance p-values were found, Tukey's post hoc test was utilized to evaluate differences across factor levels. Correlation analysis between sampling times and microbiological parameters for each station was performed using Pearson's correlation coefficient. Levene's test was firstly performed to verify homogeneity of variances. When the assumption of homogeneity of variances was not met, data were ln (x + 1) transformed to remove heteroscedasticity. nMDS analysis, ANOVA, and correlation analysis were performed considering water and sediment compartments separately and using PRIMER 6.0 and STATISTICA 10.0 softwares, respectively.

Table 1
Measured values (mean ± s.d.) of physicochemical variables, nutrients, and TRIX at the two stations in the three sampling periods.

Variable	July 2018		July 2019		July 2020	
Station	A	B	A	B	A	B
Temperature (°C)	26.4 ± 0.8	26.5 ± 1.1	27.2 ± 0.2	27.4 ± 0.6	28.0 ± 0.3	27.9 ± 0.3
Salinity (psu)	37.7 ± 0.8	37.8 ± 0.9	36.6 ± 0.3	36.8 ± 0.4	36.5 ± 0.3	36.6 ± 0.2
pH	8.0 ± 0.2	8.1 ± 0.3	8.1 ± 0.1	8.2 ± 0.4	8.0 ± 0.1	8.2 ± 0.1
N-NH_3 μM/l	0.4 ± 0.4	0.1 ± 0.1	1.3 ± 1.0	2.1 ± 0.7	2.2 ± 1.2	2.2 ± 1.6
N-NO_3 μM/l	3.2 ± 3.9	0.8 ± 0.6	0.3 ± 0.1	0.2 ± 0.2	0.4 ± 0.5	0.5 ± 0.4
N-NO_2 μM/l	0.31 ± 0.05	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.3
N_{tot} μM/l	43.3 ± 8.9	29.4 ± 6.7	13.3 ± 6.8	11.7 ± 9.7	5.6 ± 3.7	4.8 ± 2.3
P-PO_4 μM/l	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
P_{tot} μM/l	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.8 ± 0.4
TRIX	4.45 ± 1.29	2.46 ± 0.64	4.44 ± 0.07	4.14 ± 0.45	3,84 ± 0.18	4.23 ± 0.26

3. Results

3.1. Chemical-physical variables

Table 1 reports the mean values (± s.d.) of the chemical-physical variables measured in the field at the two stations A and B over the three years. Temperature ranged between the minimum value of 25.8 °C at Station B in July 2018 and the maximum value of 28.3 °C at Station A in July 2020. Salinity range was between 36.4 psu, measured in July 2019 at both stations, and 38.4 psu, measured in July 2018 at Station B. pH ranged between 7.6, measured at Station B in July 2018, and 8.3, measured at Station A in July 2019.

3.2. Nutrients and trophic status

Nutrient concentrations varied between the stations and over the years (Table 1). N-NH_3 varied between 0.01 μM/l at Station B in 2018 and 3.3 μM/l at Station B in 2020. Concerning N-NO_3, the minimum value of 0.02 μM/l was measured at Station B in 2019 and the maximum value of 7.7 μM/l was measured at Station A in 2018. N-NO_2 reached the minimum value of 0.01 μM/l at Station B in 2018 and the maximum value of 0.9 μM/l at Station A in 2018. The N_{tot} minimum value was 2.01 μM/l at Station A in 2020, while the maximum value was 50.5 μM/l at Station A in 2018. P-PO_4 ranged between 0.01 μM/l at Station B in 2019 and 2020 and 0.32 at Station A in 2018. Finally, P_{tot} range was between 0.22 μM/l at Station A in 2019 and 1.21 μM/l at Station B in 2020.

TRIX values calculated at the two stations over the three sampling periods are reported in Table 1. The index varied between 2.01 at Station B in July 2018 and 5.36 at Station A in July 2018. The highest value was recorded at Station A during the first measurement. Afterwards, TRIX decreased and the QR changed from "moderate" to "good". At the end of the monitoring period, Station B appeared quite similar to Station A.

3.3. Microbiology

Fig. 2 shows the results of microbiological analysis on sediments and seawater as regards culturable vibrios, total coliforms, fecal coliforms, and fecal enterococci at the two stations and in the three sampling periods. Results for *Escherichia coli* and *Salmonella* spp. in the sediments and the seawater are reported in Table 2. All the above-mentioned microbiological parameters were measured for the first time in July 2018, before the introduction of bioremediators. In particular, the mean concentrations of culturable vibrios in the sediment samples were 1.3×10^4 ± 100 CFU/g at Station A and 7.7×10^3 ± 256 CFU/g at Station B (Fig. 2a). With regard to seawater, culturable vibrios reached the value of 179 ± 10 CFU/mL at Station A and 177 ± 14 CFU/mL at Station B (Fig. 2b). In the same period (July 2018), total coliforms in the sediments reached the value of 230 ± 6.2 MPN/100 g at Station A and 130 ± 9 MPN/100 g at Station B (Fig. 2c). Their concentration in the seawater was 280 ± 18 MPN/100 mL at Station A and 49 ± 3 MPN/100 mL at Station B (Fig. 2d). Fecal coliform density in the sediments was 230 ± 9 MPN/100 g at Station A and 90 ± 11 MPN/100 g at Station B (Fig. 2e). While their density in the seawater was 28 ± 1.6 MPN/100 mL at Station A and 49 ± 3 MPN/100 mL at Station B (Fig. 2f). Fecal enterococci density reached the same value at both stations in the sediments (3480 ± 191.4 MPN/100 g) (Fig. 2g) as well as in the seawater (49 ± 3 MPN/100 ml) (Fig. 2h). *Escherichia coli* reached the mean value of 40.0 ± 9.4 MPN/100 g at both stations in the sediments (Table 2). In the seawater, the mean values of 33.0 ± 1.3 MPN/100 mL at Station A and 27 ± 5.3 MPN/100 mL at Station B were assessed (Table 2). *Salmonella* spp. was absent in both the sediments and the seawater, at Station A and B (Table 2).

In July 2020, two years after the placement of bioremediators at Station A, culturable vibrios density in the sediments showed a remarkable decrease at both stations (Fig. 2a). The decrease in total coliforms in the sediments at Station A was remarkable in July 2020 compared to July 2018 too (Fig. 2c). They almost completely went to zero in the seawater at both stations (Fig. 2d). Fecal coliforms showed a similar pattern to total coliforms in both the sediments and the seawater (Fig. 2e, f). The mean density of fecal enterococci in the sediment samples dropped at both stations from 3480 ± 191.4 MPN/100 g to 330 ± 11.5 MPN/100 g in July 2020, two years after the installation of IMTA-based bioremediation system (Fig. 2g). Their mean concentration in the seawater samples decreased to 17 MPN/100 mL at both stations in July 2020 (Fig. 2h). Concerning *E. coli* in the sediments, a density equal to that measured in 2018 was recorded at Station A in July 2020. At Station B, this value resulted equal to 0. Conversely, the concentration in the seawater dropped to 0 at both stations (Table 2).

Results of ANOVA test on microbiological parameters in the seawater and the sediments measured at both stations in the three sampling periods are reported in Table 3. The analysis of variance performed on water samples showed significant differences in the abundance of the examined bacteria in relation with station and time factors ($p < 0.05$). A significant interaction between stations and time factors ($p < 0.05$) was found for all the considered bacteria, except for vibrios and *E. coli* (Table 3). Post hoc comparisons revealed that the differences between stations were significant only in July 2018 and 2019, while in July 2020 the two stations showed a comparable bacterial concentration. However, the bacterial concentration showed a strong negative correlation with time for all microbiological parameters (Table 4). In the sediment samples, the station and time factors showed significant main effects and interactions for all microbiological parameters, except for *E. coli* ($p < 0.05$). Correlation analysis revealed that the abundance of bacteria in the sediment samples at Station A (except for *E. coli*) had a similar pattern to that in the water samples -decreasing significantly with time (Table 4) - while in some parameters the differences between stations remained significant even in July 2020 (Table 3). In July 2020, the abundance of vibrios and *E. coli* remained significantly higher at Station A than at Station B, whilst total and fecal coliforms resulted more abundant at Station B than at Station A.

3.4. Microtox

Interstitial water analyzed using the Microtox® test confirmed the absence of toxicity at both stations and over all the study years (Table 5).

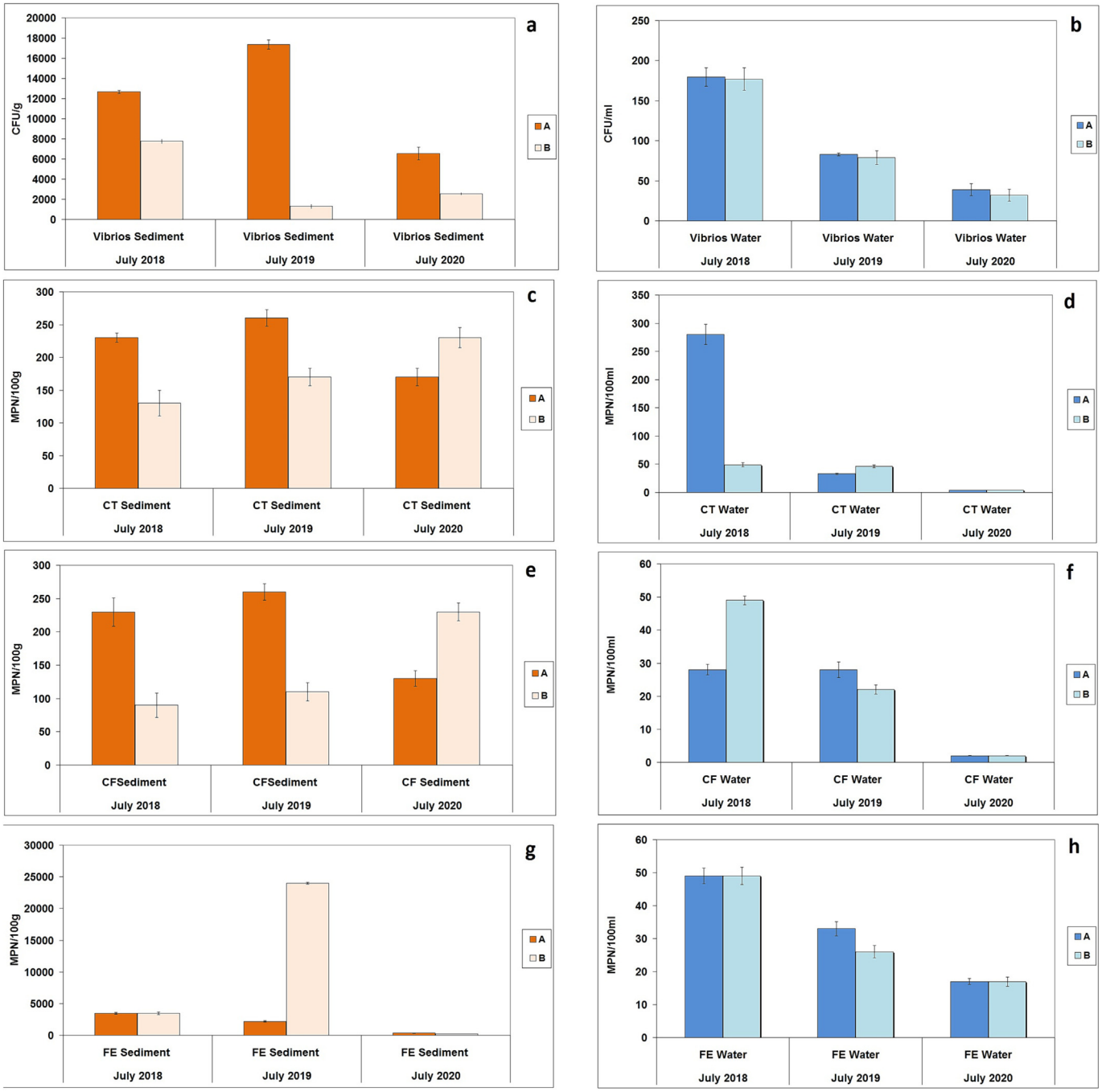

Fig. 2. Concentrations of culturable vibrios (Vibrios), total coliforms (CT), fecal coliforms (CF), and fecal enterococci (FE) in seawater and sediment samples collected in July 2018, 2019 and 2020 at the two sampling sites (A, B). Bacterial counts are reported as mean values ± S.D. ($n = 3$). Bacterial counts are expressed as: CFU/g for culturable vibrios in sediment (a), CFU/mL for culturable vibrios in seawater (b); MPN/100 g for total coliforms (c), fecal coliforms (e) and fecal enterococci (g) in sediments; as MPN/100 mL for total coliforms (d), fecal coliforms (f) and fecal enterococci (h) in seawater.

Table 2
Escherichia coli (MPN/g and MPN/100 ml) and *Salmonella* spp. (presence/absence) results in sediment and water samples at the two stations in the three sampling periods.

Variable	July 2018		July 2019		July 2020	
	A	B	A	B	A	B
Sediment						
E. coli	40.0 ± 9.4	40.0 ± 9.4	60.0 ± 5.7	0	40.0 ± 9.4	0
Salmonella spp.	Absence	Absence	Absence	Absence	Absence	Absence
Water						
E. coli	33.0 ± 1.3	27.0 ± 5.3	2.0 ± 0.8	2.0 ± 0.8	0	0
Salmonella spp.	Absence	Absence	Absence	Absence	Absence	Absence

Table 3
Results of ANOVA test on water and sediment microbiological parameters measured at both stations A and B in the three sampling periods July 2018 (T_1). July 2019 (T_2) and July 2020 (T_3).

Water		Total coliforms			Fecal coliforms			Fecal enterococci			Vibrios			*Escherichia coli*			Microtox		
Source of variation	df	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Station	1	23,762.00	3609.42	*	112.50	37.12	*	24.50	5.25	*	84.50	1.19	NS	16.96	2.35	NS			
Time	2	42,645.50	6477.80	*	2043.50	674.42	*	1560.50	334.39	*	31,785.50	448.73	*	1635.17	239.29	*			
St*Time	2	28,266.50	4293.65	*	301.50	99.50	*	24.50	5.25	*	9.50	0.13	NS	16.1	2.35	NS			
Residual	12	6.58			3.03			4.67			70.8			6.83					
Total	17																		
Tukey's test																			
St(Time)		$A(T_1) > B(T_1)$			$A(T_1) < B(T_1)$			$A(T_1) = B(T_1)$											
		$A(T_2) < B(T_2)$			$A(T_2) > B(T_2)$			$A(T_2) > B(T_2)$											
		$A(T_3) = B(T_3)$			$A(T_3) = B(T_3)$			$A(T_3) = B(T_3)$											
Time(St)		$T_1(A) > T_2(A) > T_3(A)$			$T_1(A) = T_2(A) > T_3(A)$			$T_1(A) > T_2(A) > T_3(A)$			$T_1(A) > T_2(A) > T_3(A)$			$T_1(A) > T_2(A) > T_3(A)$					
		$T_1(B) = T_2(B) > T_3(B)$			$T_1(B) > T_2(B) > T_3(B)$			$T_1(B) > T_2(B) > T_3(B)$			$T_1(B) > T_2(B) > T_3(B)$			$T_1(B) > T_2(B) > T_3(B)$					

Sediment		Total coliforms			Fecal coliforms			Fecal enterococci			Vibrios			*Escherichia coli*			Microtox		
Source of variation	df	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Station	1	8450.00	118.74	*	18,050.00	145.96	*	0.3716	899.8000	*	1.531	1470.300	*	5338.89	25.97	*	0.0304	165.9394	*
Time	2	1850.00	25.99	*	1050.00	8.49	*	3.3767	8176.1000	*	0.256	245.700	*	605.56	2.95	NS	0.0761	415.1212	*
St*Time	2	12,050.00	169.32	*	30,050.00	242.99	*	0.6453	1562.5000	*	0.349	334.900	*	1538.89	7.49	*	0.0157	85.4848	*
Residual	12	71.20			123.70			0.0004			0.001			205.56			0.0002		
Total	17																		
Tukey's test																			
St(Time)		$A(T_1) > B(T_1)$			$A(T_1) > B(T_1)$			$A(T_1) = B(T_1)$			$A(T_1) > B(T_1)$			$A(T_1) = B(T_1)$			$A(T_1) > B(T_1)$		
		$A(T_2) > B(T_2)$			$A(T_2) > B(T_2)$			$A(T_2) < B(T_2)$			$A(T_2) > B(T_2)$			$A(T_2) > B(T_2)$			$A(T_2) > B(T_2)$		
		$A(T_3) < B(T_3)$			$A(T_3) < B(T_3)$			$A(T_3) = B(T_3)$			$A(T_3) > B(T_3)$			$A(T_3) > B(T_3)$			$A(T_3) = B(T_3)$		
Time(St)		$T_1(A) < T_2(A) > T_3(A)$			$T_1(A) = T_2(A) > T_3(A)$			$T_1(A) > T_2(A) > T_3(A)$			$T_1(A) < T_2(A) > T_3(A)$						$T_1(A) > T_2(A) > T_3(A)$		
		$T_1(B) < T_2(B) < T_3(B)$			$T_1(B) = T_2(B) < T_3(B)$			$T_1(B) < T_2(B) > T_3(B)$			$T_1(B) = T_2(B) < T_3(B)$						$T_1(B) > T_2(B) = T_3(B)$		

Reported abbreviations and symbols: St station; NS not significant.
* $p < 0.05$.

Sediments always showed a TSI < 1, corresponding to an evaluation of no toxicity. However, at the beginning of the project, Station A showed the highest values.

3.5. Macrozoobenthos indices

Fig. 3 shows the variation of AMBI and M-AMBI indices over time. Initially, Station A environmental status was assessed as "poor" according to AMBI, with a value of 4.8, and "moderate" according to M-AMBI, with a value of 0.4 (Table 6). However, an amelioration of this status after the conversion of the fish farming plant into an IMTA was assessed (Table 6). The AMBI index reached a value of 2.3 and the M-AMBI index reached a value of 0.7, both indicating "good" conditions of the environmental status. At Station B, AMBI varied between 2.7 - indicating "good" environmental status - in July 2018, and 3.4 - indicating "moderate" environmental status - in July 2020. M-AMBI values ranged between 0.9 in July 2018 and 0.8 in July 2020, both indicating "high" environmental conditions.

Table 4
Results from Pearson's correlation analysis between sampling time (Time) and microbiological parameters (CT, Total coliforms; CF, Fecal coliforms; FE, Fecal enterococci; Vibrios, *E. coli*, Microtox).

Water A						
Time	CT	CF	FE	Vibrios	*E. coli*	
Pearson correlation ($N = 9$)	**−0.91**	**−0.86**	**−0.99**	**−0.97**	**−0.90**	
Water B						
Time	CT	CF	FE	Vibrios	*E. coli*	
Pearson correlation ($N = 9$)	**−0.89**	**−0.99**	**−0.96**	**−0.97**	**−0.88**	
Sediment A						
Time	CT	CF	FE	Vibrios	*E. coli*	Microtox
Pearson correlation (N = 9)	−0.65	**−0.73**	**−0.99**	−0.56	0	**−0.92**
Sediment B						
Time	CT	CF	FE	Vibrios	*E. coli*	Microtox
Pearson correlation (N = 9)	**0.98**	**0.91**	−0.13	**−0.76**	**−0.77**	**−0.84**

Significant correlation coefficients ($p < 0.05$) are given in bold.

Diversity and species richness were also lower at the beginning of the project at Station A. Before the implementation of the IMTA system, the assemblage at Station A was characterized by a low diversity and included opportunistic species that tolerate high organic loads, such as the dominant *Capitella capitata* (Fabricius, 1780). Only 8 months after the conversion of the plant, the assemblage composition at this site changed: the *C. capitata* population decreased and was replaced by species indicating good environmental conditions such as *Spiochaetopterus costarum* (Claparède, 1869). The diversity index (H′) values increased over time at Station A, while they decreased slightly at Station B during the second year and then remained almost unchanged (Table 6). Data about changes in species composition over time are available in detail in Borghese et al. (unpublished results).

3.6. Zoobenthic biodiversity on hard substrates

The species richness observed over time on artificial hard substrates represented by concrete anchoring blocks and chains for fish cage anchoring is shown in Fig. 4. At the beginning of the project, a lower number of species was recorded at Station A than at Station B (28 and 47 taxa, respectively). The artificial hard substrates under the cages were highly heterogeneous and sparsely populated by macrobenthic organisms with large portions of the substrate covered by sediments. At this stage, the community was dominated by filter feeder invertebrates with a conspicuous presence of bivalves, especially *Mytilus galloprovincialis,* and large solitary or colonial ascidians, mainly *Pyura dura* (Heller, 1877), *Styela plicata* (Lesueur, 1823), *Microcosmus* spp., *Aplidium* spp. and the *Sabella spallanzanii* polychaeta. All of them collectively accounted for about 80 % of the entire macrobenthic community. Two years after the conversion into an IMTA plant, very high values of species richness were reached at Station A (66 taxa), while a slight decrease in it was measured at Station B (34 taxa). A greater complexity was also observed in the taxa composition on the artificial hard substrates at Station A. The shift in the macrozoobenthic community, mainly due to the strong reduction in mussel number, introduced different species of sponges, colonial bryozoans, and colonial ascidians. Conversely, a rather constant community structure in

Table 5
Microtox bioassay results.

Variable	July 2018		July 2019		July 2020	
	A	B	A	B	A	B
Sediment (STI)	0.33 ± 0.01	0.13 ± 0.01	0.07 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Interstitial water (%biolumiscence inhibition)	Hormensis	Hormensis	Hormensis	Hormensis	Hormensis	Hormensis

terms of number of species and coverage was observed at Station B during the three years.

3.7. Multivariate analysis

Fig. 5 shows the dispersion of the centroid sampling stations deriving from all the measured chemical-physical and biological parameters both in the seawater (Fig. 5a) and the sediments (Fig. 5b) as overlay vectors on nMDS plot, which provides a graphical view of the global situation existing in the studied area throughout the examined periods. In particular, the analysis of water samples clearly showed that Station A and B were highly separated in July 2018. The clustering of the considered variables at Station A compared to Station B could be due to the different environmental conditions existing between the stations. By contrast, in July 2019 and July 2020 the stations became more similar. As regards sediments, Station A appeared more separated from Station B in both July 2018 and July 2019, but the differences between the two stations were smaller in July 2020. By contrast, in July 2019 and July 2020 the stations became more similar than in the starting period.

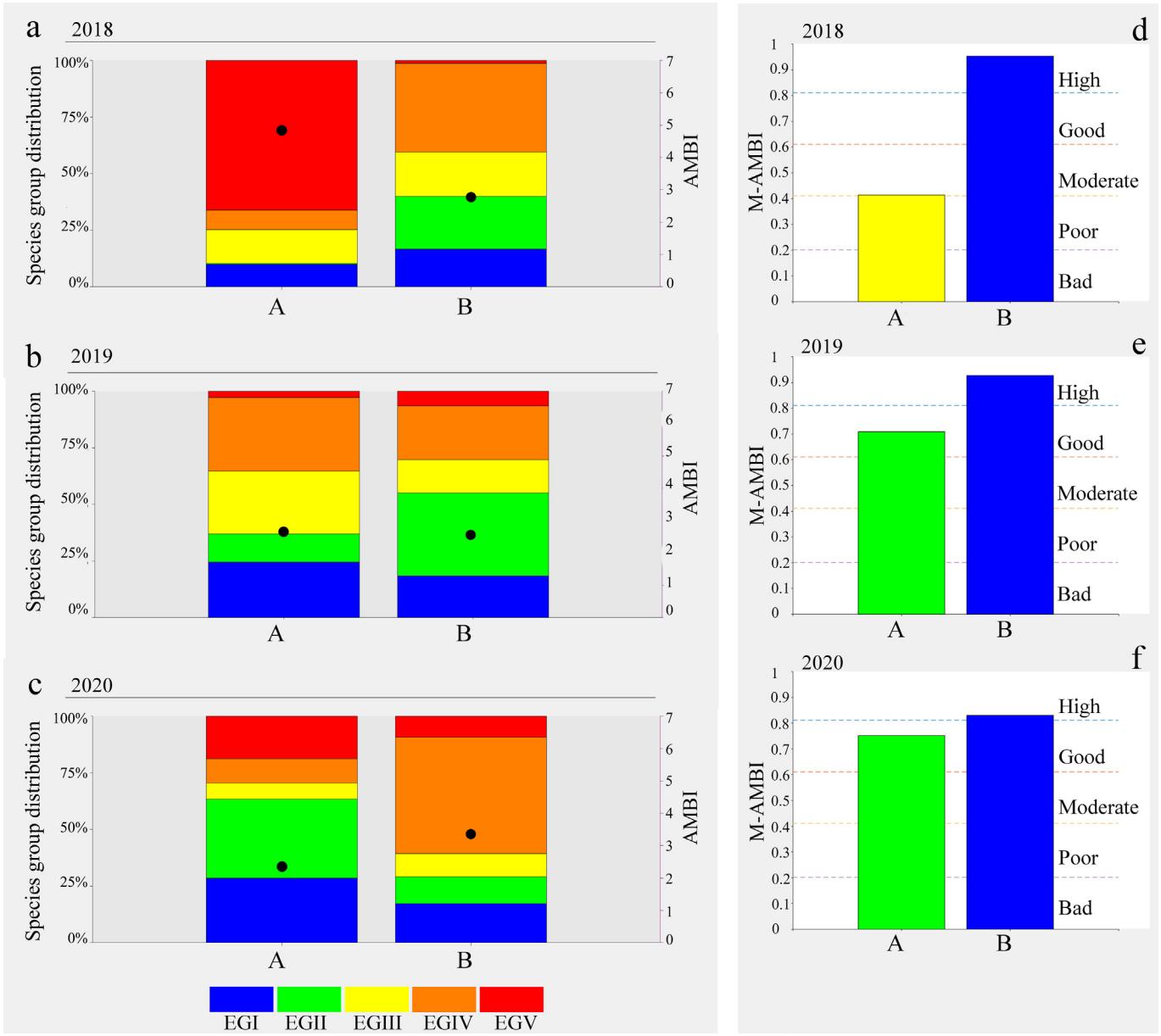

Fig. 3. Representation of AMBI (a, b, c) and M-AMBI (d, e, f) indices calculated in July in the different years (2018, 2019, 2020) at the two stations A and B.

Table 6
Values of different macrobenthos indices measured at both stations in the three sampling period. AMBI: AZTI Marine Biotic Index; H′: Shannon-Wiener diversity index; S: species richness; M-AMBI: Multivariate-AMBI.

Variable	July 2018		July 2019		July 2020	
	A	B	A	B	A	B
AMBI	4.8099	2.7766	2.6495	2.4495	2.3438	3.3547
Status	Poor	Good	Good	Good	Good	Moderate
H′	2.3222	5.1829	3.8976	4.9538	4.1884	4.4545
S	29	63	36	58	36	57
M-AMBI	0.41485	0.95327	0.70904	0.92768	0.75179	0.82982
Status	Moderate	High	Good	High	Good	High

4. Discussion

Aquaculture produces a significant amount of metabolic wastes, leading to a general deterioration of coastal ecosystems and the consequent loss of biodiversity (Chopin et al., 2001). The problem is particularly acute in farms located in enclosed areas with a reduced dispersion of the wastes, resulting in the eutrophication of the sediments and the seawater surrounding the farming cages. Recently, Remedia LIFE, a project funded under the LIFE Programme and carried out in the Mar Grande of Taranto, has led to the implementation of a pilot Integrated Multitrophic system in a small inshore fish farm. Here, for the first time, polychaetes, sponges, seaweeds, and mussels were co-farmed as bioremediators at a nearly industrial scale (Giangrande et al., 2020). As the first action of the project, Remedia LIFE monitored the plant to understand the existing environmental situation below and around the cages and to identify the most suitable area for the installation of bioremediators, that is the one most impacted by aquaculture activities (Giangrande et al., 2022). This preliminary action served as a baseline to assess the changes of the environmental quality occurring after the positioning of the selected bioremediating organisms. The measurement of biological and chemical-physical variables, along with the assessment of AMBI and M-AMBI, showed that the nutrient load was mainly concentrated in a limited area of the plant, presumably due to the main current. In this area (Station A), where the IMTA system was later put in place, a lower environmental quality was assessed. Instead, the rest of the area around the farm (Station B) was characterized by a quite good environmental quality. After placing the bioremediators, we assessed the trend of the same environmental parameters utilized in the *ex ante* monitoring comparing Station A to Station B for two years during the summer. Sampling was carried out in winter too (data not shown) and the results showed an improvement over time in the environmental quality within the fish farm, corroborating the here presented data. From our results some interesting conclusions can be inferred.

In the IMTA system, bioremediating organisms grew without any feed other than that given to the fish in the farm. In this context, the wastes from farmed fish and uneaten feed became a source of energy for all the bioremediators reared around the fish cages. Their synergistic action restored the water column transforming waste into a large amount of additional biomass, that could be used as a co-product of aquaculture (Giangrande et al., 2005; Stabili et al., 2006a, 2019). Macroalgae utilized ammonium, nitrate and phosphorous excreted by fish in the water column, simultaneously gaining nutrients for growth and removing aquaculture pollutants (Chopin et al., 2001), whilst the filter feeders, such as worms, sponges and molluscs, removed bacteria and converted significant amounts

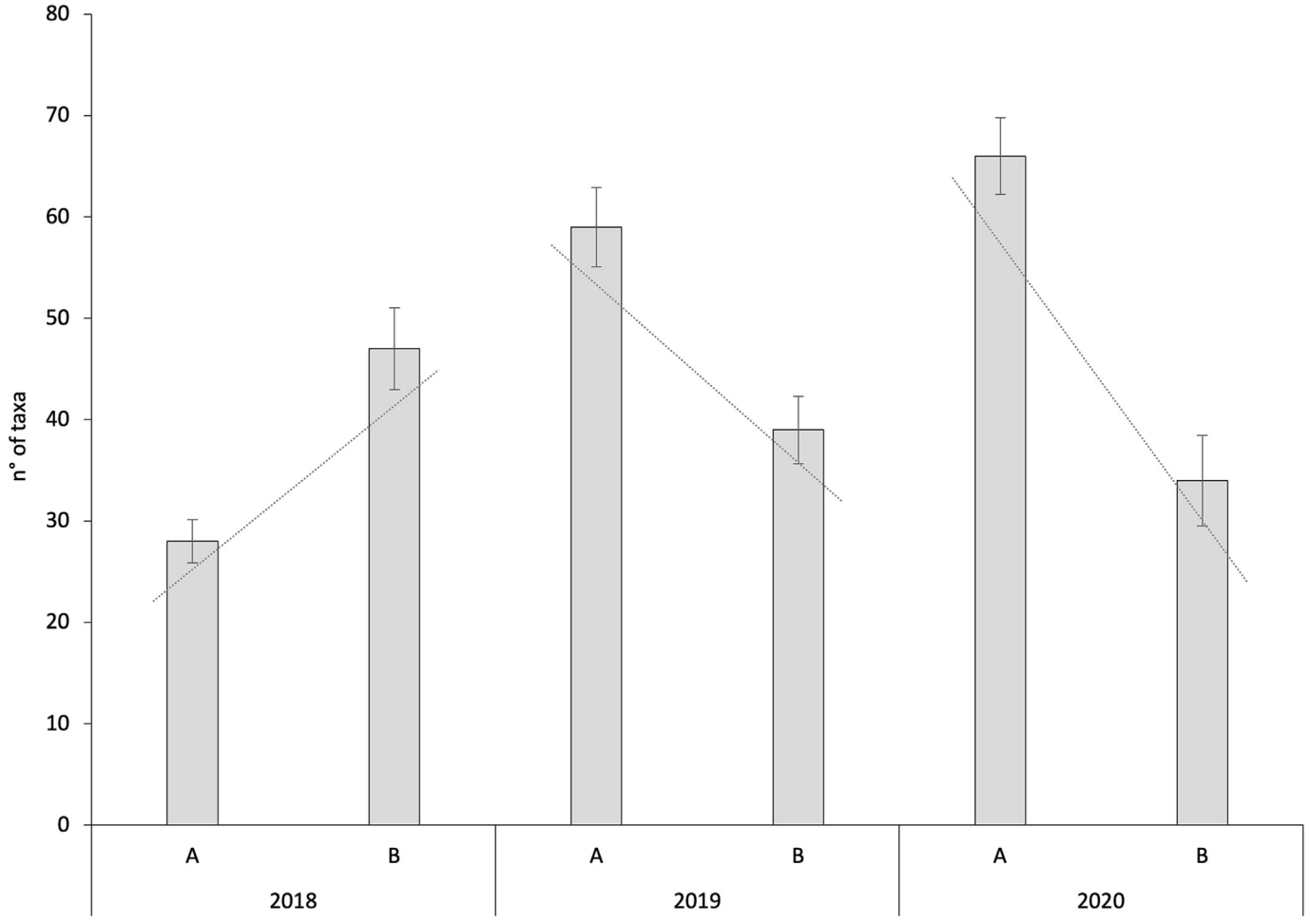

Fig. 4. Trend of species richness at the two stations on the hard bottom under the cages.

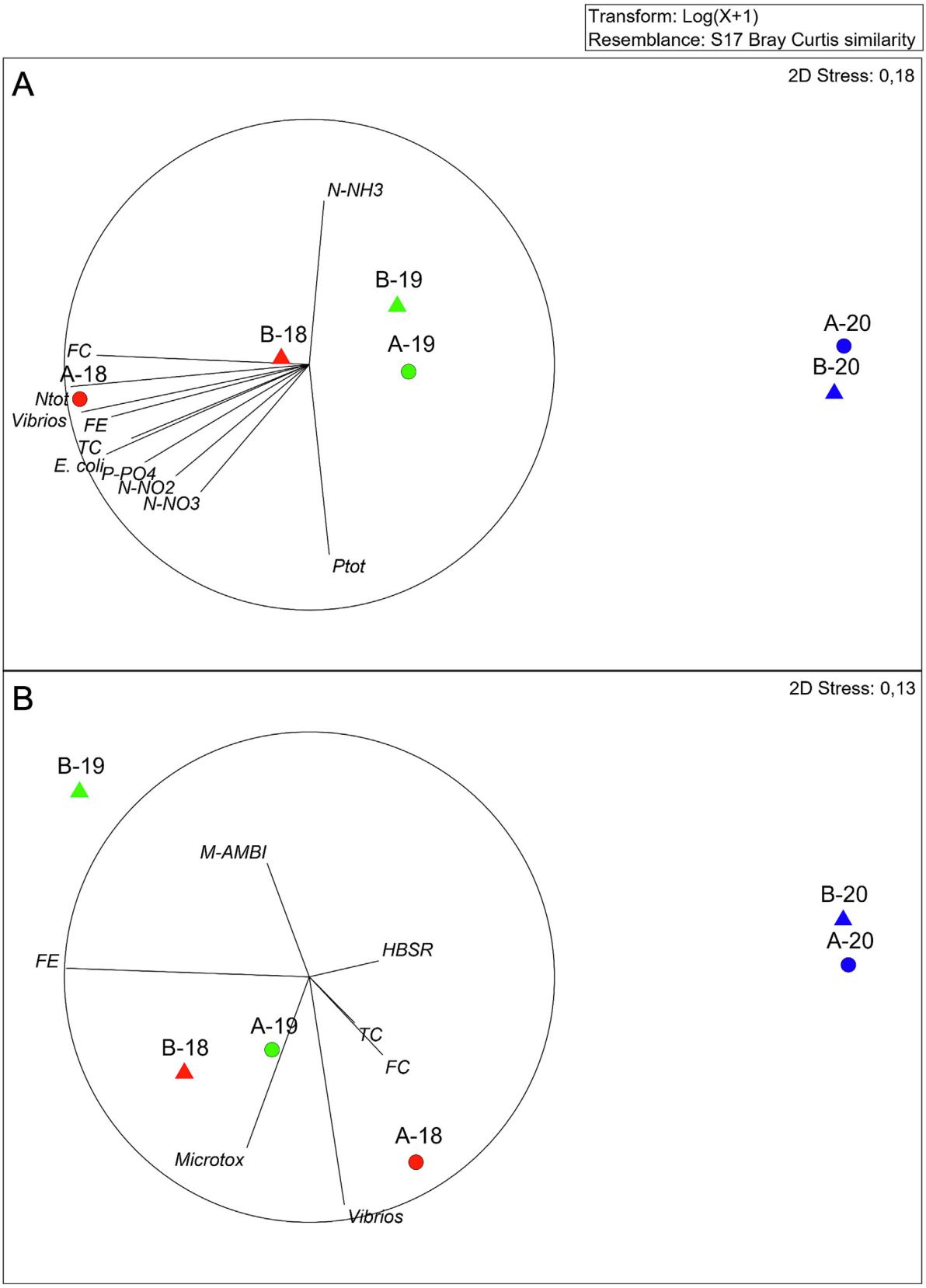

Fig. 5. Multivariate analysis on the changes over time of the chemical-physical, trophic, microbiological, and biotic measured parameters. (A) seawater column; (B) sediment, soft and hard bottom. TC = total coliforms, FC = fecal coliforms, FE = fecal enterococci, HBSR = heterotrophic bacteria.

of particulates from uneaten fish feed and faeces into harvestable body biomass. At the same time, a better ecosystem balance was achieved (Troell et al., 2003; Chopin et al., 2012). The growing performance of the organisms utilized in the IMTA system, together with the results about the obtained biomass, had been already reported by Giangrande et al. (2020).

The evident decrease in total N recorded at both stations over the years shows the effectiveness of the novel bioremediating system tested within the framework of this project. The ecological change occurred at Station A after the conversion of the plant into an IMTA system was particularly evident in the macrozoobenthic communities, which are considered the biological memory of the system. This was corroborated by the values of the biological indices assessment, suggesting an improvement of the environmental conditions. Indeed, results obtained from the comparison of macrozoobenthic assemblages in terms of species composition before and after the conversion of the plant showed that the site where the bioremediating system was placed reached an improved ecological quality status after only one year and maintained this condition over time. The combined bioremediating actions of all the filter feeders hung on the ropes of the long lines most likely supported the clearance of the water column, allowing an amelioration of the bottom benthic assemblage too (Giangrande et al., 2005; Licciano et al., 2005; Stabili et al., 2006b, 2010; Longo et al., 2010, 2016; Trani et al., 2021; Varamogianni-Mamatsi et al., 2021).

As regards microbiological standards, it should be noted that the only regulatory and legal constraints in aquaculture policies concern water quality in shellfish production areas (EU Directive 854/2004/EC, Commission Regulation (EC) 2285/2015) (EU, 2004, 2015). So far, EU seawater policy is ruled by the Water Framework Directive (2000/60/EC), which covers inland and coastal waters, and the Marine Strategy Framework Directive (2008/56/EC), which covers marine waters. In this context, in order to examine our results, the current Italian and the above cited EU regulations on the evaluation of farming localities for the cultivation of bivalves have been taken as reference in this work, as already done by Stabili et al. (2022) in a previous study in the same area. In accordance with these regulations, *Salmonella* spp. was never detected in the waters of the examined aquaculture farm. Moreover, the densities of *E. coli* as well as the other considered microbial pollution indicators resulted lower than the legal limits enforced by the aforementioned regulations, despite sampling being carried out in summer, when the densities of some microbiological parameters generally increase. As regards fecal enterococci, the evaluation of their density is not included in the current regulations for mussel culture. However, since coliforms and enterococci are often employed as indicators of fecal contamination of potable and recreational water, in the present work we have enlarged the spectrum of microbiological analyses and determined both coliforms and enterococci. The latter are indicators of older fecal contamination as they survive better in the polluted environments (Noble et al., 2004). Interestingly, a significant decrease of all the measured microbiological parameters was recorded in the seawater samples throughout the three investigated years. At Station A the concentration of total coliforms decreased from 280 ± 18 MPN/100 mL in July 2018 to 4 ± 0.2 MPN/100 mL in July 2020. A similar trend was also recorded for fecal coliforms, fecal enterococci, and *E. coli*. Furthermore, the concentration of these microbial pollution indicators became similar at stations A and B in July 2020.

The environmental effect of the selected bioremediators in reducing seawater bacterial load at Station A was observed also in the sediment samples. Indeed, the concentration of the microbial pollution indicators decreased from July 2018 to July 2020. This is noteworthy, considering that studies carried out on surface sediments of a well-established fish farm showed that benthic bacteria levels were closely related to organic enrichment and their concentration was three times higher in stations below the cages (Chávez-Crooker and Obreque-Contreras, 2010). Moreover, Vezzulli et al. (2002) observed that counts of heterotrophic bacteria indicated a shift towards Gram negative bacteria and the occurrence of pathogenic bacteria (such as *Vibrio*) in the sediments beneath fish cages. As regards vibrios in the present study, their abundance resulted strongly reduced both in the seawater and the sediment samples, thanks to the action of the selected filter feeders. Bacteria belonging to the genus *Vibrio* are of particular concern, as they are often associated with human as well as marine animals' diseases, called vibriosis, and can impact the environment and the economy. Therefore, the ecological benefit and service performed by the selected filter feeder bioremediators is crucial for human and environmental well-being. Furthermore, during the experiment, there was no impact on the toxicity of the environment. The results obtained with the Microtox® system never showed any toxicity levels for either sediment or interstitial water in all samples tested, suggesting that their quality remained unchanged. This is an interesting result, since *V. fischeri* has been shown to be a good indicator in aquaculture because it is particularly sensitive to antibiotics and disinfectants (Carballeira et al., 2018; Silva et al., 2013). Therefore, present data clearly indicate that the IMTA system represents a key factor in the development of aquaculture sustainability. The results of the present investigation were well summarized by the multivariate analysis, which indicates how the marine environment benefits from the eco-sustainable potential of the used method. The values of the parameters selected as indicators of environmental quality, such as TRIX index, AMBI, M-AMBI, microtox bioassay, microbiological analyses, and hard bottom biodiversity evaluation, show a significant amelioration of Station A two years after the bioremediating system was put in place, whilst Station B remained quite unchanged.

The environmental quality of the area under the cages, once highly degraded, was recovered with a remarkable increase in local biodiversity. Benthic biotic indices have been extensively used to assess the impact of aquaculture (Kalantzi and Karakassis, 2006; Forchino et al., 2011), as well as to detect the recovery of pristine environmental conditions after the end of the activity (Karakassis et al., 1999). However, they were never utilized in the measurement of the possible effect of the IMTA. Only measurements of organic matter have been performed to test the action of the IMTA, indicating that water quality can be sustained by IMTA activities (Mahmood et al., 2016; Ning et al., 2016). The same holds true for the TRIX index, which is considered a good index for the evaluation of the seawater trophic conditions at a European level (Pettine et al., 2007), but no published data are available about its assessment in IMTA plants besides the one implemented within the framework of Remedia LIFE. The only information available concerns the comparison of TRIX in the seawater around a fish farm (TRIX = 5.31) and at its reference station (TRIX = 4.5) in the Gulf of Trieste (Flander-Putrle and Malej, 2003). The uniqueness of the system developed within the Remedial LIFE project is that it was the first time that a new set of bioremediators that are particularly efficient in restoring the marine ecosystem was used. Moreover, our results are noteworthy because, for the first time, the bioremediation effect was measured *in situ*.

5. Conclusion

The data clearly indicate that the IMTA system integrating macroalgae and filter-feeder organisms, such as polychaetes, sponges and mussels, into a breeding site is particularly efficient at improving the environmental quality around the fish cages. In such perspective, the recovery of the polluted areas becomes consistent with the need of providing new scenarios for territorial development and improving certain social and territorial services that have not been assessed until now. The importance of ecosystem services (ES) in our millennium was already underlined by several authors (Millennium Ecosystem Assessment, 2005; TEEB, 2011; de Groot et al., 2012; Comino et al., 2014) indicating that the assessment of ES may effectively support decision makers in planning policies aimed at improving pollution remediation and, potentially, developing new local economic growth as well as social well-being (Deutsch et al., 2003; Marulli and Mallarach, 2005; Daily et al., 2009; Busch et al., 2012). In light of all this, particular emphasis has been placed on the development of sustainable approaches to coastal aquaculture (Costa-Pierce, 2002) and the promotion of ecological practices to improve ecosystem health. Therefore, considering our results, the IMTA system represents a fascinating solution to make marine aquaculture sustainable and cost-effective.

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CRediT authorship contribution statement

Loredana Stabili: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration,

Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Adriana Giangrande:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Daniele Arduini:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **Jacopo Borghese:** Data curation, Formal analysis, Investigation, Methodology, Validation. **Antonella Petrocelli:** Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Giorgio Alabiso:** Formal analysis, Investigation. **Patrizia Ricci:** Formal analysis, Investigation. **Rosa Anna Cavallo:** Formal analysis. **Maria Immacolata Acquaviva:** Formal analysis, Investigation. **Marcella Narracci:** Formal analysis, Investigation. **Cataldo Pierri:** Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **Roberta Trani:** Data curation, Formal analysis, Investigation. **Caterina Longo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Adriana Giangrande reports financial support was provided by LIFE programme.

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