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Influence of an innovative IMTA system (Mediterranean Sea, Italy) on environmental and biological parameters: Seasonal analysis

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Abstract

The influence of benthic suspension feeders as bioremediating organisms on water column seston and their ability to mitigate fish farm waste was assessed by monthly analyses for one year. A monthly monitoring from July 2020 to October 2021 of physico-chemical and biochemical variables of the water column and the sediment was performed in an in-shore mariculture plant located in the Mar Grande of Taranto (Italy), comparing two adjacent areas that differ in type of farming. The first (reference site) is a fish monoculture, while the second consists of an innovative Integrated Multi-Trophic Aquaculture system (IMTA site). The annual cycle of chlorophyll-*a* concentration showed values corresponding to mesotrophic/eutrophic conditions, with peak production in early fall (4.64 µg L^{-1}). During the spring and summer sampling times, when the amount of aquaculture waste is greater than during the rest of the year, the reference site showed the highest lipid (151.73 µg L^{-1}) and organic matter (189.00 mg g^{-1}) values with respect to those at the IMTA site. The IMTA bioremediating organisms possibly acted as underwater gardens changing the seston quality and availability and providing protection and food for the zooplankton community, which showed higher abundance of individuals for most sampling times at the IMTA site, peaking during September and October in both 2020 and 2021 (max 232,985.20 ind m^{-3}). Our results demonstrate 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.

Keywords: water column seston; benthic suspension feeders; fish farm; long-term analysis; waste mitigation.

1 Introduction

In the last 60 years, the global consumption of food from aquatic sources increased at an average annual rate of 3%, about twice as fast as the annual growth rate of the global population (1.6%) (FAO, 2022). Aquaculture has recently overtaken fishery as the largest producer of seafood products for human consumption and consequently the development of innovative methods to manage some of its environmental impacts (e.g., release of nutrients in the environment) has received more attention (FAO, 2022; Karakassis et al., 2000; Mazzola and Sarà, 2001). Even if the replacement of wild stock fisheries by aquaculture sounds positive, several factors have to deeply change to render it sustainable and respectful of the environment (Hishamunda et al., 2009).

The Integrated Multi-Trophic Aquaculture (IMTA) system has been developed to improve sustainability in intensive aquaculture facilities, combining fed aquaculture species with additional commercially relevant organisms, capable of extracting organic and/or inorganic compounds from the surrounding seawater (fish farm waste), creating a balanced system to promote environmental sustainability (biomitigation), economic stability (product diversification), and social acceptability (better management practices) (Biswas et al., 2020; Chopin et al., 2001; Giangrande et al., 2020;

Neori et al., 2004; Troell et al., 2009). Most of these IMTA installations are based on two or four well known generally edible organisms, such as fishes, seaweeds and bivalves (FAO, 2022), but other options, including sponges, ascidians and polychaetes (benthic suspension feeders), are possible and profitable (Giangrande et al., 2020; Gökalp et al., 2019; Ju et al., 2015). Such suspension feeding organisms can form a highly biodiverse marine forest, enhancing species interaction, complexity and biodiversity (Rossi et al., 2017a).

The physiological and ecological characteristics of the bioremediating organisms determine the rate of capture and assimilation of particles or nutrients in the water column and consequently the bioremediation capacity of the IMTA system (Lamprianidou et al., 2015). The presence of benthic organisms, particularly suspension feeders, is known to be an important factor affecting near bottom seston composition and concentration (Rossi and Gili, 2005, 2009). Indeed, the efficiency of IMTA is related to the selection of reared species, belonging to different trophic levels, as well as the amount of filter feeder organisms acting on the system that capture detritus and the microbial components of the water column (filter feeder invertebrates), or of those species that assimilate dissolved nutrients (algal component; Granada et al., 2016). The variability in suspension feeding strategies may lead to a higher particle spectra filtration (Rossi et al., 2017b), as suspension feeding organisms have a wide size range of particle capture (Gili and Coma, 1998).

It is well known that there is high environmental and biological variability in coastal ecosystems (especially in the Mediterranean Sea), and the main interactions between physical and biological factors follow seasonal trends in temperate seas, with pulses of peak production in spring and autumn (Coma and Ribes, 2003; Estrada, 1996; Ribes et al., 1999; Rossi and Gili, 2005). In these periods, the primary and secondary productivities fuel the benthic suspension feeding organisms that store lipids for reproduction (Rossi et al., 2017b). However, the environments affected by finfish mariculture discharges, especially from in-shore facilities located in enclosed areas with limited waste dispersion, are exposed to a mixture of seston supplemented with daily fish wastes, which could influence the seasonal quantity and quality of seston. Such practices increase the risk of eutrophication of the area particularly during the summer period, when farmed fish growth is higher and intensive feeding occurs (Neofitou and Klaoudatos, 2008).

The present study refers to the innovative in-shore IMTA rearing model, performed within the EU REMEDIA Life project (LIFE16 ENV/IT/000343) in the Gulf of Taranto (Ionian Sea), where filter feeder bioremediating organisms, such as polychaetes, sponges and mussels, coupled with macroalgae and the natural fouling assemblages, have been reared within a fish farm for the first time in Europe (Giangrande et al., 2020).

The bioremediation effects of this IMTA system on benthic assemblages, the planktonic bacterial communities and the capture of microplastic have been already evaluated (Borghese et al., 2023; Fraissinet et al, 2024; Stabili et al*.*, 2023), highlighting an environmental improvement related to the IMTA system. However, the integration of quantitative information on water column dynamics on a long-term basis would provide a more complete picture of the system, remarking the importance of these bioremediating organisms as cleaners, but also understanding their impact on the water column seston, highlighting trends in the seasonal interactions between physico-chemical (i.e., environmental) and biological factors.

Hence, trends of environmental variables, the quantification of organic matter in the sediment and seston composition at two different depths (1 m from the sea surface and 1 m from the bottom), were investigated through monthly analyses (from July 2020 to October 2021). Two different zones were considered: 1) the plant where the IMTA system has been implemented and 2) the area where fish monoculture is still present. Among all the seston variables, lipids, which are one of the more reliable for the calculation of available food in organic components of seston (Grémare et al., 1997), and the planktonic component (chlorophyll-*a* concentration and zooplankton composition and abundance) have been monitored in the water column to assess temporal and spatial differences. The comparison of all measured variables between the multi-trophic and traditional aquaculture sites has been used to assess the potential environmental effects of the bioremediating organisms.

2 Materials and methods

2.1 Study area

The study area (Fig. 1) is located on the south-west side of the Mar Grande of Taranto (40°25'56'' N; 17° 14'19'' E) (Southeast Italy, Ionian Sea). The Mar Grande of Taranto is a semi-enclosed basin reaching a maximum depth of 45 m. The local water surface current direction is north-east to south-west at a speed of ~3 cm s^{-1}. At the bottom, the direction of the current is inverted, proceeding from south-west to north-east at a speed of ~1.3 cm s^{-1} (Giangrande et al., 2020).

The investigation was performed at the aquaculture plant "Maricoltura Mar Grande", which covers a surface of 0.06 km^2 and is positioned at about 600 m from the coast. It consists of 15 cages (Ø 22 m), working at a depth ranging from 7 to 12 m and producing about 100 tons $year^{-1}$ of European seabass *Dicentrarchus labrax* (Linnaeus, 1758) or sea bream *Sparus aurata*, Linnaeus, 1758. The density and developmental stage of the reared fish remained similar in all cages throughout the time of the experiment.

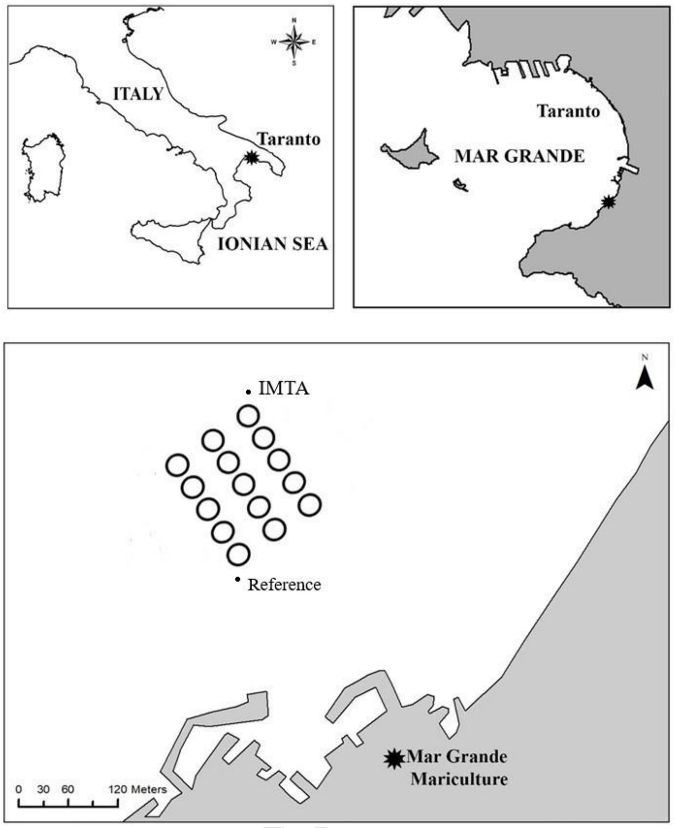


Fig. 1. Study area. Black dots: IMTA site and reference site. ✸: Mar Grande Mariculture plant; **O**: fish cages.

At the beginning of the REMEDIA Life project (2019), part of the plant (6 cages) has been converted into an IMTA system, resulting in the formation of two distinct areas within which the respective sampling sites were established: a reference site (Ref), where the monoculture persisted; and the IMTA site, treated with the bioremediating organisms (Fig. 2).

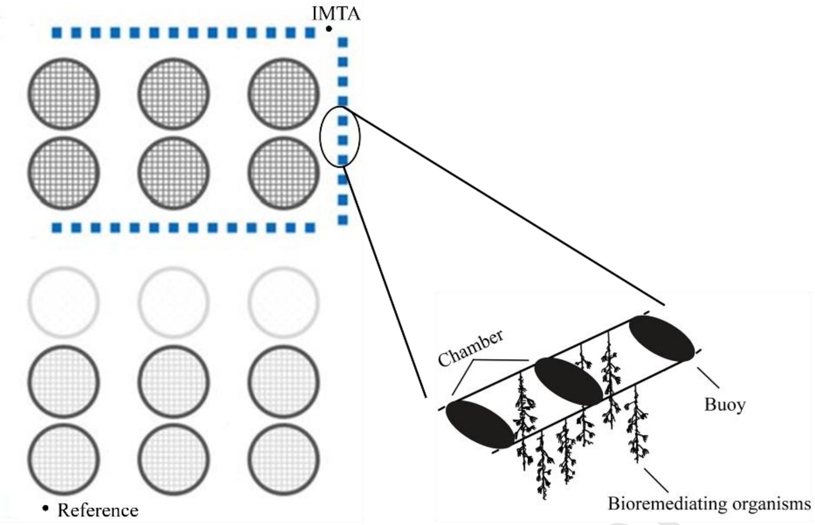


Fig. 2. Black dots: sampling sites. IMTA site on the upper right, reference site on the lower left. Blue squares: arrangement of the breeding chambers within the long lines.

The IMTA system was placed in the northernmost part of the plant, where the farm wastes accumulate, according to the hydrodynamic regime of the area (Giangrande et al., 2022). The IMTA bioremediating system, utilizing the polychaete *Sabella spallanzanii* (Gmelin, 1791), the sponge *Sarcotragus spinosulus* Schmidt, 1862, the mollusc *Mytilus galloprovincialis* Lamarck, 1819 and the macroalgae *Chaetomorpha linum* (O.F.Müller) Kützing, 1845 and *Gracilaria bursa-pastoris* (S. G. Gmelin) P.C. Silva, 1952, is described in detail in Giangrande et al. (2020).

The REMEDIA Life IMTA plant, consisting of about 400 collectors, was placed around six cages. The system was supported by buoys creating three long lines in which the space between two consecutive buoys constituted a breeding "chamber" hosting module, represented by different typologies of collectors to house the reared organisms: vertical bare collectors for the recruitment of polychaetes, modules with explant of sponges and horizontal collectors with explant of macroalgae. In each chamber, several 5 m long nets containing molluscs were also placed alternating with polychaetes and sponges in order to fill empty gaps. Macrofouling collectors consisted of coconut fiber ropes, 2 cm wide and 10 m long. The sponge-rearing modules were 7 m long ropes in which sponge explants were inserted within plastic nets at regular intervals every 40 cm. Macroalgae were instead placed at the surface within plastic socks. Many other organisms that are part of the fouling community have grown on the collectors, increasing the filtering capacity of the system.

2.2 Sampling activities

Several physico-chemical and biochemical variables were monitored approximately each month in the IMTA and in the reference sites, at two different depths: 1 m from the sea surface (surface = s) and 1 m from the bottom (depth = d), for a total of 13 samplings, from July 2020 to October 2021 (Table 1). Sampling activities were based on depth and area: IMTA (s); IMTA (d); Ref (s) and Ref (d). Samples were taken at two different depths to get a more complete picture of the water column for each site. Sampling at one meter above the seabed is considered a standard to sample the water column, away from the small turbulence of the upper water layers (Thomsen, 1999). The measurements have been carried out around 11:00 AM for each sampling site.

Table 1. Sampled variables and number of replicates made each time in the sampling area. Chl-*a* = chlorophyll-*a*; Zoop = zooplankton; OM = organic matter.

	Environmental variables	Chl-*a*	Lipids	Zoop. abundance	OM
N° of replicates	Single measurement	3	3	3	3
N° of stations	2	2	2	2	2
Sampling period	Jul 20-Oct 21	Jul 20-Oct 21	Jul 20-Oct 21	Jul 20-Oct 21	Jul 20-Oct 21
N° of samplings	13	13	13	13	13
N° of depths	2	2	2	Whole water column	Sea bottom

2.3 Environmental variables

The measurement of the physico-chemical variables was carried out using a multiparameter probe (IDROMAR, IP050D, San Giuliano Milanese, Italy) for the evaluation of pH, dissolved oxygen (DO), and temperature (T).

2.4 Biochemical variables

Water samples were collected through Niskin bottle (ABTNK-02, 2.5 L, Aquatic BioTechnology, Cádiz, Spain) and placed into PVC containers. The samples were stored in ice (6-10 ºC) and in the dark until arrival at the laboratory.

To determine the chlorophyll-*a* (chl-*a*) concentration three 200 mL replicates for each site, depth, and sampling time were filtered through GF/F pre-combusted (450 ºC, 5 h) glass fibre filters. Filters were stored at -20 ºC. Chl-*a* was extracted in 8 mL 90% acetone in the dark at 4 ºC for 24 hours. The supernatant was read in a spectrophotometer at three different wavelengths: 630, 663 and 750 nm and chl-*a* concentrations were calculated according to the spectrometric equations reported in Jeffrey and Humphrey (1975): chl-a = 11.43 E_{663} – 0.64 E_{630}, where E_x denotes the extinction coefficient at wavelength x. The absorbance at 750 nm was subtracted from those two wavelengths to give the turbidity-corrected value.

To analyse total lipids of particulate suspended matter, three 1500 mL replicates for each site, depth, and sampling time were filtered through pre-combusted (450 ºC, 5 h) GF/F filters. Total lipids were determined using Barnes and Blackstock (1973) spectrophotometrical (colorimetrical) procedure, slightly changed as by Rossi and Fiorillo (2010). Filters were extracted in chloroform-methanol (2:1 v/v). The extract was dried, then sulphuric acid and vanillin were added to complete the colorimetric method. Blanks were made to control the interference of filter glass fibre particles and a calibration line was made using cholesterol as standard (Grémare et al., 1997). Colorimetric measurements were read at 520 nm using a spectrophotometer (UV Mini1240, Shimadzu). Results are expressed in µg L^{-1}.

2.5 Organic matter content

For the organic matter (OM) content, three replicates of the upper layer (3 cm) of the soft bottom sediment were collected for each site, depth, and sampling time in PVC containers (12 cm^3) via scuba diving. Samples were stored in ice (6-10 ºC) until arrival at the laboratory, where they were stored at -20 ºC. Samples were dried (60 °C, 48 h), sieved (mesh size of 200 µm), placed in crucibles, weighed and ignited in a muffle furnace (450 ºC, 5 h). Samples were weighed again to measure the inorganic component, and the organic matter was obtained from the difference between the two weight measurements. Percentage weight loss was calculated for each replicate.

2.6 Zooplankton concentration

Water samples were collected through a plankton net (mesh size of 80 µm). Each sampling was conducted by vertical towing along the entire water column (from ~10 m depth to the surface) to prevent interference of species vertical partitioning. The volume of water filtered during each tow, estimated with a flow meter (HYDRO-BIOS Model 438115) placed at the net mouth, ranged from 0.113±0.006 m^3 to 0.905±0.045 m^3. Samples were stored in 90% ethanol solution and the specimens were identified and counted under an inverted microscope.

2.7 Statistical analysis

The variability in chl-*a* and lipid concentrations was assessed in the IMTA and in the reference sites at two different depths [1 m from the sea surface (surface = s), and 1 m from the bottom (deep = d)] and 13 sampling points (from July 2020 to October 2021, Table 1) by permutational analyses of variance (PERMANOVA, Anderson, 2001). The design consisted of three factors: Sampling Time (ST, as a random factor with 13 levels), Depth (DE, as a fixed factor with 2 levels) and Treatment (TR, as a fixed factor with 2 levels) with n = 3. Replicate samples were randomly selected within each treatment. In addition, organic matter in sediments was assessed at 13 sampling times according to the following two-factor design: Sampling Time (ST, as a random factor with 13 levels), and Treatment (TR, as a fixed factor with 2 levels) with n = 3. PERMANOVA analyses were performed based on Euclidean distances of previously normalized data, using 999 random permutations of the appropriate units (Anderson and Braak, 2003). When significant differences were encountered ($P < 0.05$), post-hoc pairwise tests were carried out in order to ascertain the consistency of the differences across the conditions tested. Because of the restricted number of unique permutations in the pairwise tests, p-values were obtained from Monte Carlo tests. The analyses were performed using PRIMER v. 6 software (Anderson and Braak, 2003) including the PERMANOVA + add-on package (Clarke and Gorley, 2006; Anderson, 2008). Pearson’s correlation matrices and p-values were calculated using the STATISTICA software.

3 Results

3.1 Environmental variables

Water temperature (T) values showed no marked differences between sites throughout the seasonal cycle, ranging from 13.9°C, in March 2021, up to 28.3°C in July 2020 (Fig. 3). The thermocline formation was observed in the period between May and July in both sites, intensifying the stratification and hindering vertical diffusion. For the rest of the year the water column temperature was almost homogenous.

The recorded values of pH ranged from a minimum of 7.85 in February 2021 up to a maximum of 8.26 in October 2021 (Fig. 3). No clear trend was observed seasonally.

The recorded values of dissolved oxygen (DO) ranged from 3.15 ppm in October 2020 up to 8.10 ppm in June 2021 (Fig. 3). Higher DO values were measured during periods of colder water temperatures, from November 2020 to June 2021.

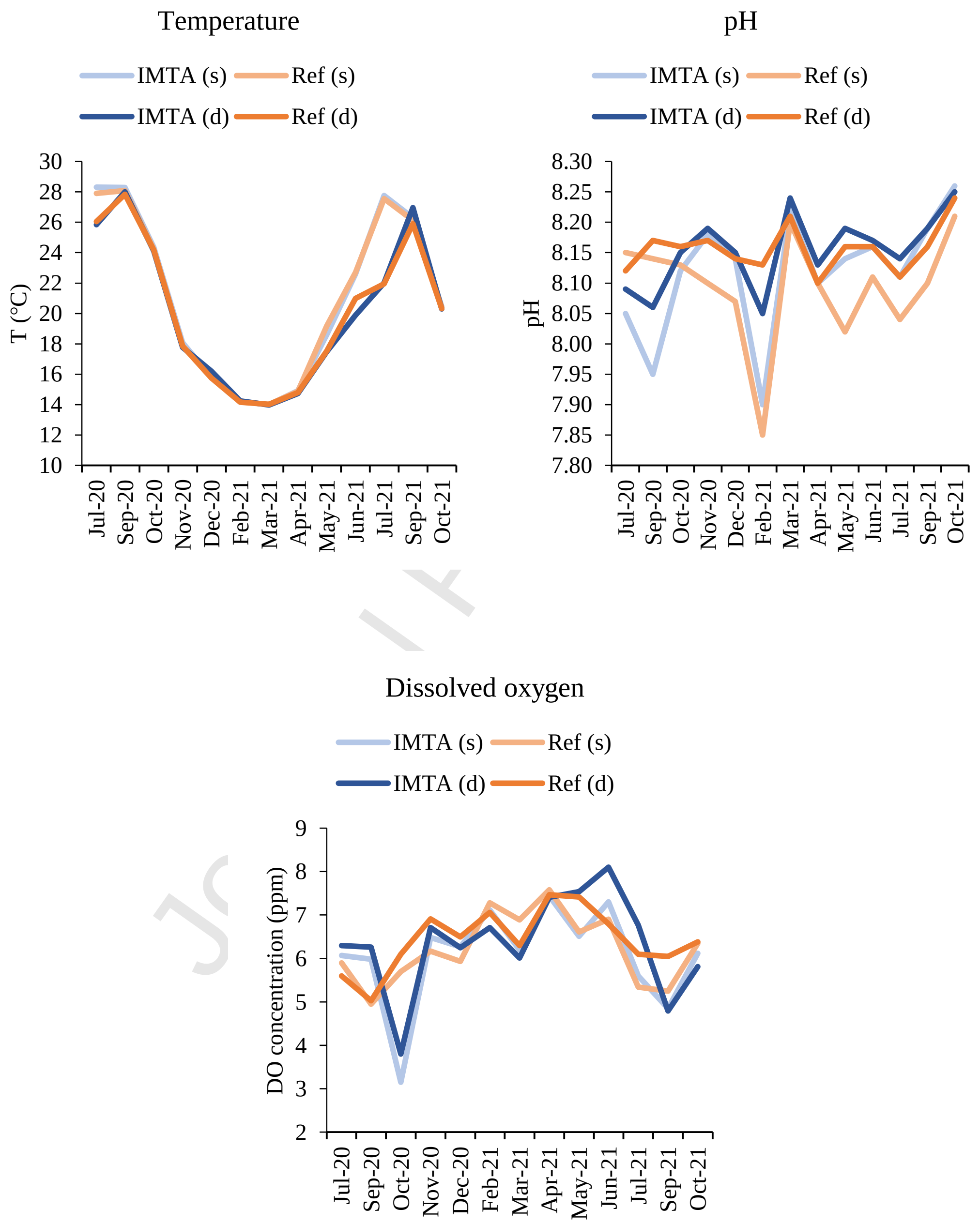


Fig. 3. Seasonal trend of temperature; pH and dissolved oxygen concentration in the IMTA and reference sites. Ref = reference; (s) = surface sites; (d) = depth sites; T = temperature; DO = dissolved oxygen.

3.2 Biochemical variables

The recorded average values of chl-*a* ranged from 0.25 µg L^{-1} up to 4.64 µg L^{-1} (Fig. 4) and showed, as a general trend, higher overall values in warmer periods. Differences of chl-*a* contents were found between IMTA (d) and Ref (d), where in October and November 2020 the Ref (d) site showed significant higher values than IMTA site (October, t = 18.018, $P < 0001$; November, t = 6.202, $P < 0.01$; see Table 3), while the significant lower values were observed in April 2021, (t = 4.659, $P < 0.01$; see Table 3).

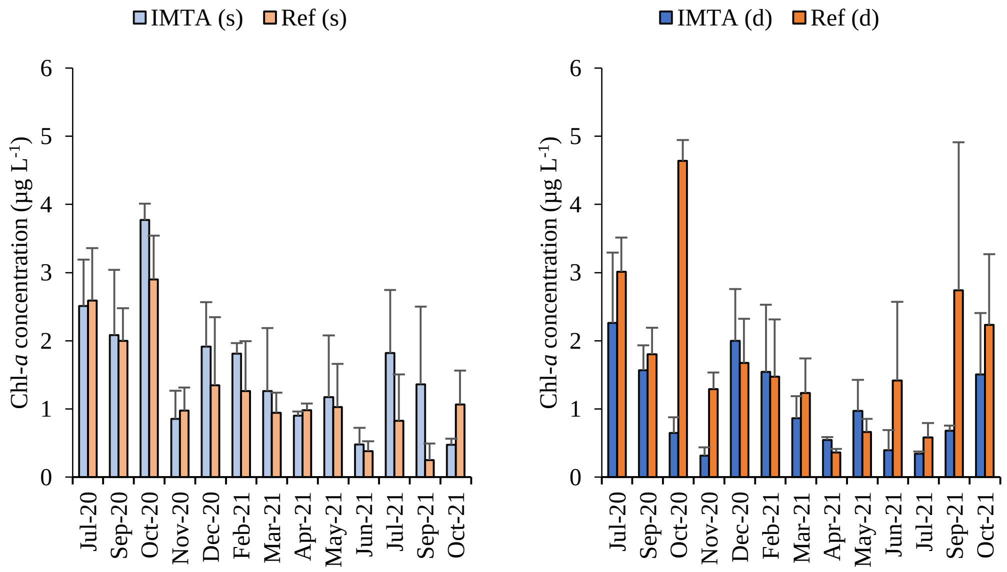


Fig. 4. Seasonal trend of chlorophyll-*a* concentration in the IMTA and reference sites. On the left: IMTA and reference surface sites; on the right: IMTA and reference depth sites. Ref = reference; Chl-*a* = chlorophyll-*a;* (s) = surface sites; (d) = depth sites.

The recorded average lipid concentration values ranged from 0.00 µg L^{-1} up to 151.73 µg L^{-1} (Fig. 5). On average, higher values were found at both the depth sites. Significant higher values in lipid concentration values were observed at Ref (d) than IMTA (d) both in July (t = 6.136, $P < 0.01$; Table 3) and September 2021 (t = 8.890, $P < 0.001$, Table 3) and at Ref (s) than IMTA (s) in September 2021 (t = 3.959, $P < 0.05$, Table 3). The highest values measured in March/April 2021 did not show significant differences among IMTA and Ref sites (Table 3). Lipid concentration was found to be lower at the IMTA than Ref site during June at both depths (shallow, t = 4.908, $P <$

0.01; deep, t = 8.010, $P < 0.01$; Table 3),and in May (t = 4.000, $P < 0.05$; Table 3) and October 2020 (t = 4.732, $P < 0.01$; Table 3) at shallow seawaters.

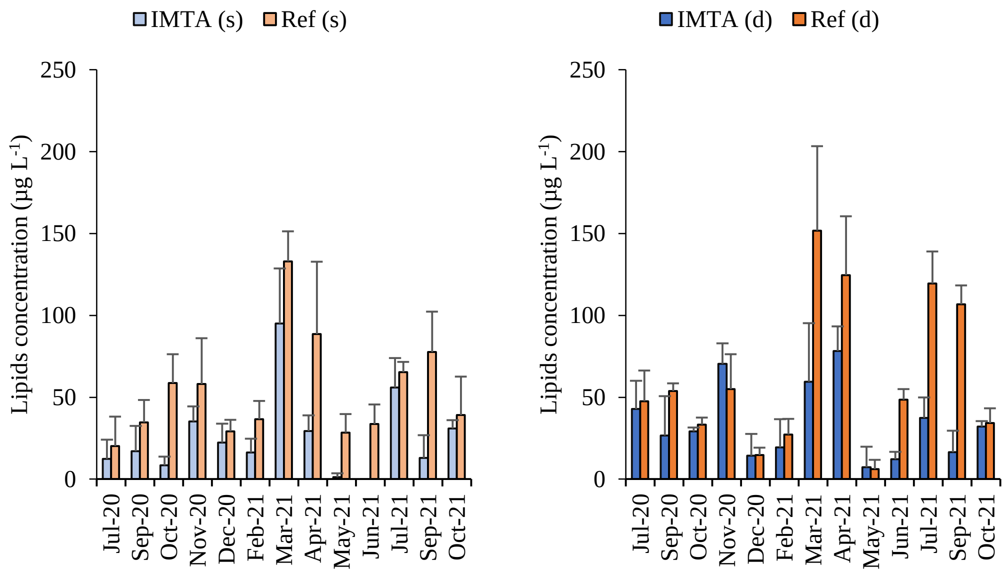


Fig. 5. Seasonal trend of lipids concentration in the IMTA and reference sites. On the left: IMTA and reference surface sites; on the right: IMTA and reference depth sites. Ref = reference; (s) = surface sites; (d) = depth sites.

3.3 Organic matter content

The recorded average values of the OM content present in the sediments below the fish cages ranged from 56.33 mg g^{-1} to 189.00 mg g^{-1} (Fig. 6). Significant higher values in organic matter were found at Ref site than IMTA site during July 2020 (t = 3.332, $P < 0.05$; Table 6) and June 2021 (t = 2.863, $P < 0.05$; Table 6), while lower values were observed in November (t = 3.158, $P < 0.05$; Table 6).

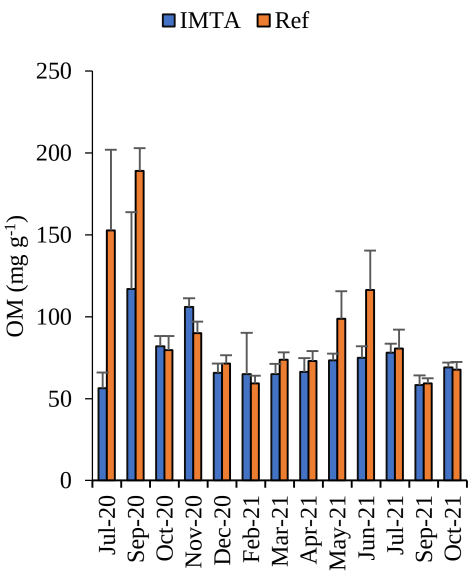


Fig. 6. Seasonal trend of organic matter concentration in the IMTA and reference sites. Ref = reference; OM = organic matter.

3.4 Zooplankton analysis

The zooplankton abundance ranged from 871.6 ind m^{-3} to 232,985.2 ind m^{-3} (Fig. 7). The seasonal trend showed higher values of abundance at the IMTA site, with highest peaks during September and October both 2020 and 2021.

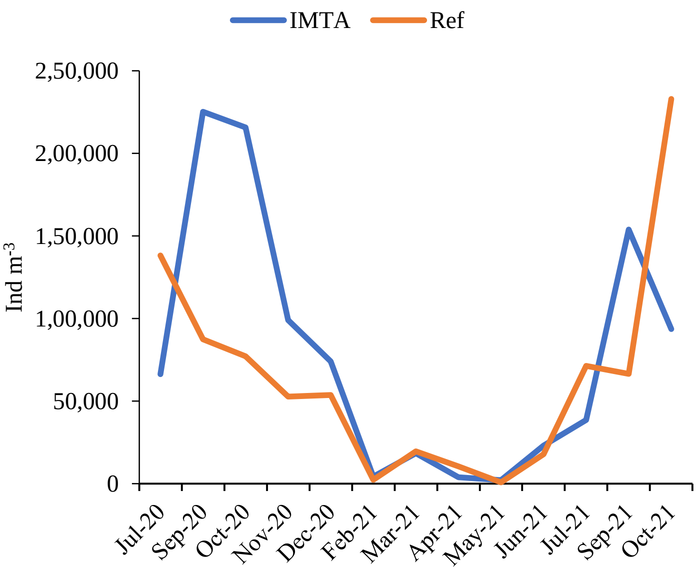
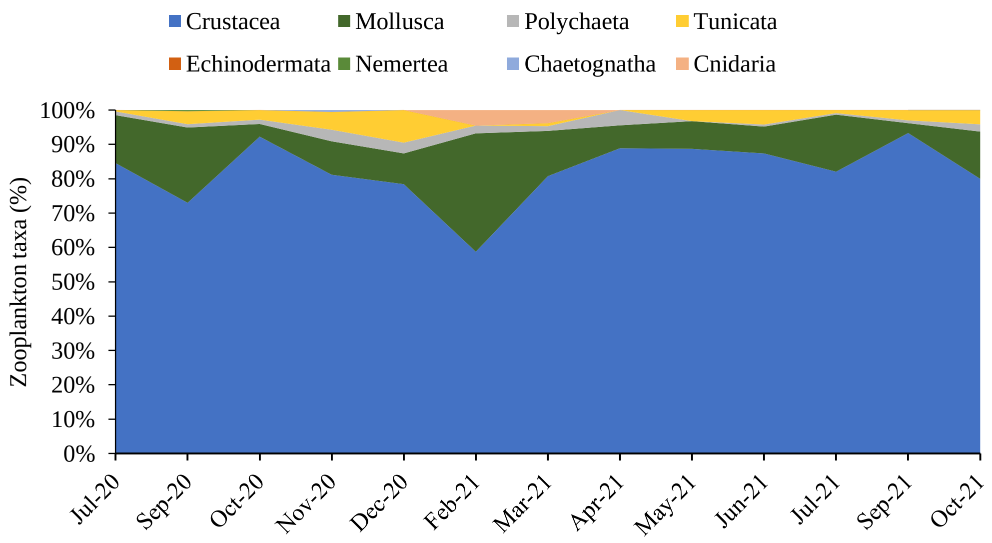


Fig. 7. Zooplankton abundance in the IMTA and reference sites. Ref = reference site; Ind = individuals.

Zooplankton composition showed similar percentages of taxa present among sites, with crustaceans representing the most abundant taxon, comprising 60% to 90% of the population for each sampling (Fig. 8).

(A) IMTA



(B) Ref

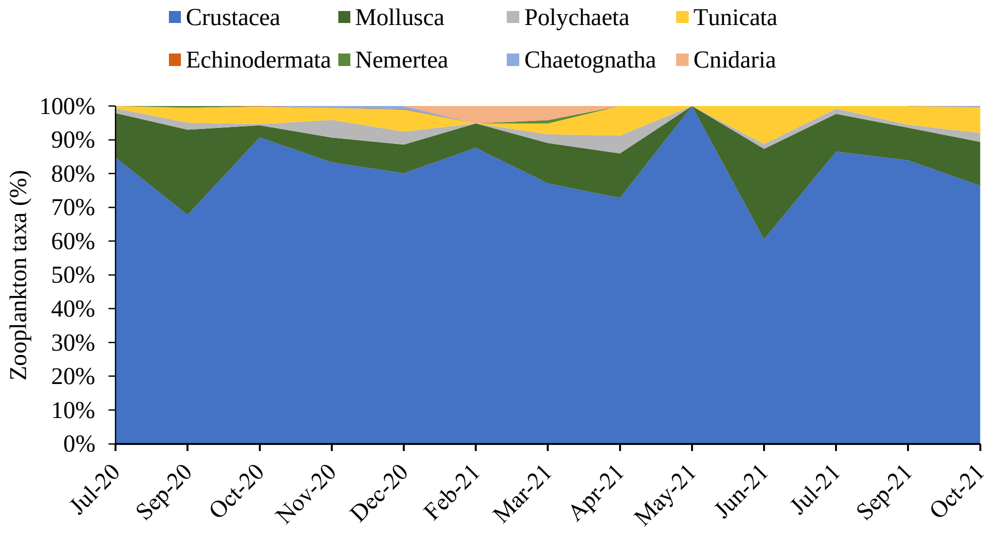


Fig. 8. Seasonal trend of zooplankton taxa percentage at: (A) the IMTA site and (B) the reference site. Ref = reference site.

3.5 Statistical analysis

The results of the PERMANOVA tests revealed that both chl-*a* and lipid concentrations varied significantly between IMTA and Ref at different depths across sampling times as underlined by the significant STxDExTR interaction term (Table 2). Post-hoc pairwise tests carried out separately within each sampling time and investigated depth showed significant differences in chl-*a* concentration among IMTA (d) and Ref (d) sites in ST3 (October 2020), ST4 (November 2020) and ST8 (April 2021) (Table 3), while significant differences in lipid concentration were found among IMTA (s) and Ref (s) sites in ST3 (October 2020), ST9 (May 2021), ST10 (June 2021) and ST12 (September 2021) and among IMTA (d) and Ref (d) sites in ST10 (June 2021), ST11 (July 2021), ST12 (September 2021) (Table 3). A significant negative correlation was reported between DO and chl-*a* concentration at (s) sites and between DO and T at (s) and (d) sites. In addition, weak positive correlations were observed between chl-*a* concentration and temperature (T) at (s) and (d) sites, while a negative correlation was found between lipid concentration and T at (s) sites (Table 4).

Table 2. Results of PERMANOVA testing for the effects of IMTA on the chlorophyll-*a* and lipid concentration at different depths across the sampling times. ST = Sampling Time; DE = Depth; TR = Treatment; df = degree of freedom; MS = mean squares; Pseudo-*F* = *F* critic; *P*(perm) = permutational level of probability; *= $P < 0.05$; **= $P < 0.01$; *** = $P \leq 0.001$; ns = not significant.

		Chlorophyll-*a* concentration			Lipid concentration		
Source	df	MS	Pseudo-*F*	*P*(perm)	MS	Pseudo-*F*	*P*(perm)
ST	12	5.493	13.825	***** 0.001**	6.536	28.808	***** 0.001**
DE	1	0.002	0.003	0.959 ns	2.082	3.845	0.074 ns
TR	1	1.618	1.985	0.177 ns	21.938	17.163	**** 0.003**
STxDE	12	0.884	2.225	*** 0.011**	0.541	2.386	0.01 ns
STxTR	12	0.815	2.051	*** 0.029**	1.278	5.634	***** 0.001**
DExTR	1	9.147	6.612	*** 0.029**	0.005	0.009	0.932 ns

STxDExTR	12	1.383	3.482	***** 0.001**	0.593	2.614	**** 0.004**
Res	104	0.397			0.227		
Total	155						

Table 3. Results of the pairwise tests contrasting chlorophyll-*a* and lipid concentration between IMTA *vs.* reference at different depths across several sampling times. ST = Sampling Time; DE = Depth; *P*(MC) = probability level after Monte Carlo simulations; t = pairwise tests; * = $P < 0.05$; ** = $P < 0.01$; *** = $P \leq 0.001$; ns = not significant.

	Chlorophyll-*a* concentration		Lipid concetration	
	IMTA *vs.* reference		IMTA *vs.* reference	
	t	*P*(MC)	t	*P*(MC)
ST1DE1	0.137	0.891 ns	0.634	0.549 ns
ST1DE2	1.135	0.352 ns	0.327	0.769 ns
ST2DE1	0.138	0.903 ns	1.469	0.216 ns
ST2DE2	0.772	0.512 ns	1.920	0.128 ns
ST3DE1	2.202	0.084 ns	4.732	**** 0.010**
ST3DE2	18.018	***** 0.001**	1.455	0.249 ns
ST4DE1	0.383	0.715 ns	1.353	0.240 ns
ST4DE2	6.202	**** 0.006**	1.069	0.352 ns
ST5DE1	0.822	0.463 ns	0.883	0.418 ns
ST5DE2	0.565	0.619 ns	0.038	0.980 ns
ST6DE1	1.279	0.280 ns	2.525	0.078 ns
ST6DE2	0.094	0.935 ns	0.684	0.496 ns
ST7DE1	0.570	0.620 ns	1.718	0.177 ns
ST7DE2	1.055	0.333 ns	2.547	0.061 ns
ST8DE1	1.192	0.279 ns	2.260	0.088 ns
ST8DE2	4.659	**** 0.008**	2.048	0.096 ns
ST9DE1	0.230	0.841 ns	4.000	*** 0.021**

ST9DE2	1.078	0.338 ns	0.151	0.880 ns
ST10DE1	0.608	0.592 ns	4.908	** **0.009**
ST10DE2	1.487	0.214 ns	8.010	** **0.003**
ST11DE1	1.499	0.231 ns	0.849	0.437 ns
ST11DE2	1.959	0.130 ns	6.136	** **0.002**
ST12DE1	1.658	0.163 ns	3.959	* **0.025**
ST12DE2	1.643	0.174 ns	8.890	*** **0.001**
ST13DE1	2.033	0.110 ns	0.595	0.575 ns
ST13DE2	0.915	0.408 ns	0.392	0.704 ns

Table 4. Pearson’s correlation matrix for chl-*a* and lipid concentration and related environmental variables (T, DO, pH) at (s) and (d) sites. Number of samples = 78; Chl-*a* = chlorophyll-*a*; T = temperature; DO = dissolved oxygen; * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

	(s)				(d)			
	Chl-*a* (µg L^{-1})	Lipids (µg L^{-1})	T (°C)	DO (ppm)	Chl-*a* (µg L^{-1})	Lipids (µg L^{-1})	T (°C)	DO (ppm)
Lipids (µg L^{-1})	-0.21 ns				-0.12 ns			
T (°C)	***0.29**	***-0.26**			****0.32**	-0.12 ns		
DO (ppm)	****-0.47**	0.13 ns	****-0.62**		-0.17 ns	-0.01 ns	****-0.56**	
pH	-0.13 ns	0.19 ns	0.06 ns	-0.20 ns	-0.04 ns	0.01 ns	-0.14 ns	-0.06 ns

The organic matter contents in the sediments varied throughout the sampling times and treatment (IMTA and Ref; Table 5). Post-hoc pairwise tests showed significant differences in OM content among IMTA and Ref in ST1(July 2020), ST4 (November 2020) and ST10 (June 2021) (Table 6). Pearson’s correlation matrices for chl-*a*, lipids OM and environmental variables near the sea bottom at (d) are reported in Table 7. Organic matter was positively correlated to T and, to a lesser degree, negatively to DO. A negative correlation was evidenced between DO and T and a positive correlation was found between chl-*a* and T (Table 7).

Table 5. Results of PERMANOVA testing for the effects of IMTA on the OM in sediments across the sampling times. OM = organic matter; ST = Sampling Time; TR = Treatment; df = degree of freedom; MS = mean squares; Pseudo-*F* = *F* critic; *P*(perm) = permutational level of probability; *** = $P \leq 0.001$; ns = not significant.

OM				
Source	df	MS	Pseudo-*F*	*P*(perm)
ST	12	3.405	13.803	*** **0.001**
TR	1	5.703	3.885	0.070 ns
STxTR	12	1.468	5.950	*** **0.001**
Res	52	0.247		
Total	77			

Table 6. Results of the pairwise tests contrasting organic matter in sediments within IMTA *vs*. reference at different depths across several sampling times. OM = organic matter; ST = Sampling Time; *P*(MC) = probability level after Monte Carlo simulations; t = pairwise tests; * = $P < 0.05$; ns = not significant.

OM		
	IMTA *vs*. reference	
Groups	t	*P*(MC)
ST1	3.332	* **0.039**
ST2	2.551	0.063 ns
ST3	0.383	0.730 ns
ST4	3.158	* **0.024**
ST5	1.281	0.287 ns
ST6	0.385	0.701 ns
ST7	1.949	0.121 ns
ST8	1.120	0.327 ns
ST9	2.522	0.056 ns

ST10	2.863	* __0.035__
ST11	0.361	0.716 ns
ST12	0.265	0.825 ns
ST13	0.413	0.709 ns

Table 7. Pearson’s correlation matrix for organic matter in the sediment and related environmental variables (T, DO, pH). Number of samples = 78; OM = organic matter; Chl-*a* = chlorophyll-*a*; T = temperature; DO = dissolved oxygen; * = $P < 0.05$; ** = $P < 0.01$; ns = not significant.

	OM (mg)	Chl-*a* (μg L^{-1})	Lipids (μg L^{-1})	T (°C)	DO (ppm)
Chl-*a* (μg L^{-1})	0.07 ns				
Lipids (μg L^{-1})	0.03 ns	-0.12 ns			
T (°C)	__**0.40__	__**0.32__	-0.12 ns		
DO (ppm)	__*-0.23__	-0.17 ns	-0.01 ns	__**-0.56__	
pH	-0.08 ns	-0.04 ns	0.01 ns	-0.14 ns	-0.06 ns

Zooplankton abundance was found to correlate positively with T, and negatively with DO (Table 8).

Table 8. Pearson’s correlation matrix for zooplankton abundance and related environmental variables (T, DO, pH, OM, chl-*a*, lipids). Number of samples = 78; Ind = individuals; T = temperature; DO = dissolved oxygen; Chl-*a* = chlorophyll-*a*; OM = organic matter; * = $P < 0.05$; ** = $P < 0.01$; ns = not significant.

	Ind m^{-3}	T (°C)	pH	DO (ppm)	Lipids (μg L^{-1})	Chl-*a* (μg L^{-1})
T (°C)	__**0.63__					
pH	0.06 ns	-0.14 ns				
DO (ppm)	__**-0.61__	__*-0.57__	-0.13 ns			
Lipids (μg L^{-1})	-0.16 ns	-0.12 ns	-0.04 ns	0.00 ns		
Chl-*a* (μg L^{-1})	0.19 ns	0.24 ns	-0.05 ns	-0.19 ns	-0.24 ns	
OM (mg)	0.20 ns	__**0.40__	-0.08 ns	__*-0.22__	0.03 ns	0.07 ns

4 Discussion

The Mediterranean is generally defined as an oligotrophic sea characterized by low natural nutrient concentrations (Tanhua et al., 2013). Seasonal studies have shown that the Mediterranean is more productive at certain times of the year, particularly in the coastal and littoral zones (D'Alcalà et al., 2004; Lazzari et al., 2012; Rossi and Gili, 2005). Such productivity is essential to understand the response of secondary production, such as zooplankton concentration or the energy storage and reproduction of benthic suspension feeders (Calbet et al., 2001; Rossi et al., 2006).

Seasonal patterns of nutrient concentrations in the water column could be altered by human activities, particularly in-shore fish farming, as the release of nutrients from mariculture facilities is a continuous year-round process, reaching maximal values during the summer (Neofitou and Klaoudatos, 2008; Pitta et al., 1998). Such continuous release of nutrients and detritus may have consequences for the surrounding habitat, sometimes impacting benthic communities (Mayr et al., 2014).

The results of the physico-chemical and biochemical analyses described in the present work showed consistent values with respect to studies previously conducted in the Mar Grande of Taranto as well as in other areas of the western Mediterranean influenced by mariculture activities (in terms of water column variables, Belmonte et al., 2013; Giangrande et al., 2022; Soriano-González et al., 2019; Stabili et al., 2023) The Gulf of Taranto is a highly impacted area, not only due to massive mariculture activities (mainly fishes and mussels), but also because of an intense commercial, industrial and urban development (Buccolieri et al., 2006).

Regarding physico-chemical characteristics, the water temperature showed expected seasonal trend, with differences between depths in the warmer moments of the sampling period, where there was greater oxygen consumption, indicating a negative correlation between T and DO (Pearson's correlation, r = -0.62, $P < 0.01$, n = 78 at surface sites; r = -0.56, $P < 0.01$, n = 78 at depth sites; Table 4). Such results are related to the increase in oxygen consumption by biological respiration and to the bacterial activity, responsible for degrading part of the organic matter and consuming the available oxygen (Jacquet *et al*., 2021). The pH values did not show a significant correlation with the other investigated variables. In general, a slight tendency to acidification of the impacted waters due to biological activity of the farmed species has been observed globally, especially in more confined and shallow environments (Sarà, 2007). However, it seems that pH values are more dependent on intrinsic morphological and hydrodynamic features of the water bodies (depth, water movements, etc., Sarà, 2007). Since the pH may affect the phytoplankton cell growth (Johnson et al., 2022) further studies are needed to quantify composition and abundance of each phytoplankton group and clarify the relation between pH changes and primary productivity in the traditional and IMTA plants.

The annual cycle of chl-*a* concentration showed values corresponding to mesotrophic/eutrophic condition (Beiras, 2018) throughout the year, with peak production in early fall at both the depth and surface sites. The chl-*a* concentration was correlated to temperature seasonal cycles, while no relevant impact of pH on the primary productivity has been observed here (Table 4). Peaks of chl-*a* are found in the Mediterranean Sea when the water starts to be stratified in late winter-spring (Estrada and Berdalet, 1998), which is in line with the present results. However, the continuous release of nutrient excretion from reared fish may have influenced the chl-*a* values recorded, fuelling the microbial communities of the water column. In this case, the significant differences between IMTA (d) and Ref (d) (Table 3) may be due to the synergistic activity of invertebrate bioremediating organisms, capable of filtering out the phytoplanktonic component. Also, we suggest that the reared macroalgae in the IMTA facilities is directly taking up dissolved phosphorus and nitrogen, reducing the risk of local eutrophication (Wu et al., 2015). Indeed, the greatest abundance of zooplanktonic organisms in the IMTA site that feeds on the phytoplankton may also be responsible for the observed chl-*a* concentrations. Thus, what we see is probably a synergy of three factors affecting the chl-*a* levels: 1) the presence of filter feeders capable of capturing different components of the seston, including phytoplankton; 2) the presence of macroalgae in the facilities, which limits the uptake of the phytoplankton cells; and 3) the higher abundance of zooplankters that are grazing on the seston. The IMTA has thus a triple effect on the abundance of the chl-*a*, and on the abundance of phytoplankton, as on other seston components (see below).

No significant difference in chl-*a* values between the surface sites was observed (Table 3), suggesting that environmental factors such as solar irradiance may have a greater influence than bioremediating and zooplankton organisms on the concentration of chl-*a* at surface level. The positive correlation between chl-*a* and T (Pearson's correlation, $r = 0.29$, $P < 0.05$, $n = 78$ at surface sites; $r = 0.32$, $P < 0.01$, $n = 78$ at depth sites; Table 4) confirms that chl-*a* pigment concentration is a convenient index of phytoplankton biomass and water temperature is a fundamental factor influencing phytoplankton growth (Solanki et al., 2008). Such phytoplankton will be boosted by a higher nutrient concentration, which may come from the fish activity in terms of food not consumed by the fishes and related waste production. On the surface, the influence of the benthic suspension feeders may be less significant because of their lower biomass concentration, so the phytoplankton may not be controlled by filter feeding organisms.

Most of the biomass of the reared bioremediating organisms was estimated to belong to *S. spallanzanii*, with an average annual production of ~800 kg in the IMTA facility (REMEDIA Life project observation). This polychaete has a broad range of trophic plasticity and may consume both phytoplankton and organic materials from the water column (Giangrande et al., 2005). Moreover, the pseudo-faeces are compacted with the mucus during tube building, ensuring their complete removal from the system (Giangrande et al., 2005). Laboratory experiments indicated a high nutrients removal efficiency of this species (~40%) with increased activity in the warm periods (Clapin, 1996; Giangrande et al., 2005), suggesting a possible influence on the composition of the water column seston for bioremediation purposes (Giangrande et al., 2005). This is in line with the chl-*a* depletion observed in the depth IMTA site, so the impact here may be considered relevant.

In addition, OM and lipids in the water column were mechanically trapped by the support system (coconut rope collectors) where the bioremediating organisms grow, and it has been estimated that ~600 kg of mud was subtracted from the water column (during an annual production cycle) in the IMTA facility, beyond the OM captured by the worms and all the other reared organisms (Giangrande et al., 2020). Such a “sediment trap” enhances the viability of other organisms, such as deposit feeders and detritivores, in the complex three-dimensional alive structure composed of these sabellids (and other sessile organisms).

Thus, the significant differences between the sites in both lipid concentration at both depths and in the concentration of OM in the sediment, reflecting what is occurring in the water column, are possibly related to the action of the bioremediating organisms, feeding on a wide range of particles from bacteria to detritus (Coma et al., 2001). Indeed, during spring and summer, when the amount of feed spilled and consequently the plant waste is greater than during the rest of the year (Neofitou and Klaoudatos, 2008), the IMTA system can take advantage of the greater filtering activity of the bioremediating organisms, mitigating the large daily waste release. As a result, at these sampling times the reference site showed the highest lipid and OM values compare to those at the IMTA site. These results are in line with a previous study of the benthic community, which has reported an improvement in the environmental quality status of the IMTA site since its creation in 2018 (Borghese et al., 2023). A negative correlation was found between OM and DO (Pearson’s correlation, r = -0.23, $P < 0.05$, n = 78; Table 7), probably due to eutrophication processes that increase the amount of available nutrients, giving rise to high oxygen demands through respiration processes, as previously explained for the relationship between T and the DO (Bonsdorff et al., 1997).One interesting result is the potential effect of the IMTA on the zooplankton. The IMTA site showed overall higher zooplankton abundance compared to the reference site, especially in the late summer-autumn period of 2020 (when zooplankton may be abundant in Mediterranean coastal areas, Calbet et al., 2001). However, differently to other Mediterranean areas, where two zooplankton abundance peaks are reported per year (e.g., Kamburska and Fonda-Umani, 2009), Belmonte et al. (2013) highlighted that in the Taranto Sea the spring peak is negligible and maximum abundance is recorded once a year in late summer–early autumn, as confirmed by our results.

The fouling community settled on the collectors is likely to include organisms, such as hydrozoans, that feed by capturing the mesozooplankton component. However, the concentration of these passive suspension feeders that rely on zooplankton to feed (Gili et al., 1998) is not as abundant as those of other active suspension feeders. The main biomass present on the collectors belongs to the reared target organisms, which prefer the phytoplankton, detritus, bacteria and the dissolved organic matter (Coma et al., 2001, Giangrande et al., 2020). It is thus reliable to think that zooplankton will not be affected by the filtering activity of these organisms (ascidians, polychaetes, bivalves and sponges), taking advantage of the complexity of the system in terms of ecosystem engineering species (Rossi et al., 2017a).

It is known that zooplankton abundance could raise due to changes in local hydrodynamics more than a possible attraction by chemical cues, and the farms' structural components, including nets, mooring systems and tons of cultivated fish, alter the local oceanographic dynamics by reducing current velocity, favouring the retention of particles like plankton (Fernandez-Jover et al., 2016; Klebert et al., 2013; Madin et al., 2010). Therefore, it is possible that the hundreds of vertical collectors of the IMTA system may have further contributed to increase the complexity of the system by enhancing particle retention and trophic interactions, acting similarly to kelp forests, which are characterized by high abundance of zooplankton (Pakhomov et al., 2002). In the case of the marine animal forests (i.e., three-dimensional alive structures dominated by sessile metazoans, Rossi et al. 2017a), the particle retention and the zooplankton abundance is observed within the canopy (Frutos et al., 2017; Guizien and Ghisalberti, 2015), thanks to the turbulence produced by the complex structures of the suspension feeding organisms. In fact, the community of a well-structured IMTA system in the Mediterranean Sea can be defined as an animal forest, providing protection, food, and avoiding drift of the zooplankton.

5 Conclusions

Significant differences were found between sites at several samplings in lipid, chl-*a* and OM values. In particular, the lipid content of the water, which may represent the direct source of the plant's waste, was found to be lower in the water column of the IMTA system, showing the effectiveness of this system in mitigating the negative impact of fish farming. The influence of the IMTA system on seston composition and zooplankton abundance revealed mitigation as well as restoration scenarios, with potential carbon sequestration as an added benefit. The changeover from in-shore fish farms, which cause environmental pressure, to well-designed IMTA systems, coupled with the creation of underwater gardens or animal forests, can then lead to the restoration of the habitat as a whole.

Further studies at different time scales are needed to better appreciate seasonal and daily trends of the environmental variables in other plants and environmental conditions, as well as to understand whether a generalization of the bioremediation patterns is possible.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights:

- Bioremediating organisms influenced seston concentrations,
- results support the sustainability of the IMTA system,
- IMTA system mitigated fish farm waste, related to lipid concentration,
- significant differences were found in seston concentration between sites,