



Exploring the potential effects of IMTA on water column seston through intensive short-time cycles approach

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ABSTRACT

Short-time cycles of the water column can reflect undetectable changes in seasonal cycles, providing a high temporal resolution for quantifying seston availability.

Here, trends in seston quantity and quality were investigated through intensive temporal cycle assessments at an IMTA site and a nearby fish farm facility (control site) during two different periods of the year (July 2021 and March 2022). Chlorophyll-*a* values (max 10.28 $\mu\text{g L}^{-1}$) showed no significant differences between sites, while lipid values (max 394.87 $\mu\text{g L}^{-1}$) were significantly lower at the IMTA site than at the control site during most sampling times, suggesting that the bioremediating organisms at the IMTA site may help mitigate the impact of fish farming.

The IMTA site structured a marine animal forest capable of increasing the abundance of zooplankton, reaching 109,021 ind m^{-3} .

The short-time cycle appeared useful for detecting changes that occur at high temporal resolution, underlying the effects of mitigation measures behind the high variability of marine coastal systems.

1. Introduction

Aquaculture will replace an important part of the world-wide fisheries (especially the more destructive ones), now nearly equal in terms of extracted biomass (FAO, 2022). However, the monoculture of certain species may be harmful to local ecosystems (Ottinger et al., 2016). The impacts of inshore mariculture are well known and relate primarily to the release of nutrients and particulate organic matter into the water column and their consequent accumulation in the nearby sediments (Wang et al., 2017; Wang et al., 2020). The development of more sustainable farming technologies, such as the Integrated Multi-Trophic Aquaculture (IMTA) system, aims to mitigate local eutrophication by combining fish farmed species with additional commercially relevant organisms that act as bioremediators (Giangrande et al., 2020). Such organisms (algae, suspension feeders, deposit feeders, grazers, etc.) can

extract organic and/or inorganic compounds from the seawater or from the sediments affected by the excess of detritus or dissolved nutrients (Chopin, 2012; Neori et al., 2004; Troell et al., 2009). Among these organisms, benthic filter feeders may act as bioremediators, filtering a wide spectrum of particles (Coma et al., 2001; Gili and Coma, 1998). In fact, the presence of filter feeding organisms is known to be an important factor affecting seston composition and concentration in the water column (Rossi et al., 2017), as they can intercept particles and deplete the near bottom seston (Rossi and Gili, 2009). The composition of the water column is variable, and in warm temperate seas oscillates between productive and nonproductive periods (Rossi and Gili, 2005), making it difficult to discern using only a seasonal (e.g., monthly) approach (Rossi and Gili, 2007).

Seasonal trends in warm temperate seas, such as the Mediterranean, depend on the interaction of biological and physical factors, with pulses

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of peak production in spring and early autumn (Coma and Ribes, 2003; Estrada, 1996; Rossi and Gili, 2005). The water column affected by finfish mariculture discharges, however, is exposed to a mixture of seston supplemented with daily fish waste (Casadevall et al., 2021). This is especially evident in inshore facilities located in enclosed areas with limited waste dispersion, which could influence the seasonal quantity and quality of seston (Neofitou and Klaoudatos, 2008). Such continuous feeding increases the risk of eutrophication in the area, particularly during the summer period, when farmed fish growth is higher and intensive feeding occurs (Neofitou and Klaoudatos, 2008). Considering that the summer in pristine Mediterranean environments is the least productive period due to the strong water stratification (D'Alcalà et al., 2004; Estrada, 1996), it is interesting to understand the seston dynamics in an impacted aquaculture area with and without an IMTA experimental protocol based on benthic suspension feeding organisms.

The short-timescale changes in seston concentration and quality can be a tool to detect smaller scale variability in ecosystems that is undetectable with seasonal sampling designs and can certainly help provide a more complete picture of the system under consideration (tidal area, Fegley et al., 1992; upwelling area, Rossi and Fiorillo, 2010; warm temperate sea, Rossi and Gili, 2007; Antarctic, Rossi et al., 2013). Indeed, to assess the influence of environmental factors on biological and biochemical variability in the seston, short-timescale observations are required to understand the potential impact of sudden changes in seston quantity and quality (Tilstone et al., 2000), since the concentration of biochemical variables such as chlorophyll-*a* (chl-*a*) or lipids can drastically change in only few hours (Rossi and Fiorillo, 2010; Rossi and Gili, 2007), as well as the zooplankton abundance and composition (Barange and Gili, 1988; Orejas et al., 2013). Short-time cycles (i.e., high temporal resolution sampling, observing changes every few hours) are thus considered essential to understand the potential energy inputs in benthic assemblages (Rossi and Gili, 2007), serving as a potential cue to better understand altered systems such as aquaculture facilities. Indeed, aquaculture environments often undergo rapid fluctuations in water quality parameters, such as nutrient levels, dissolved oxygen, and particulate matter (Zang et al., 2011). Short-term sampling may help researchers detect these changes and understand the direct effects of aquaculture activities. Frequent data collection could also assist in assessing the environmental impacts of IMTA operations, providing insights into how different trophic levels respond dynamically. Additionally, understanding short-term variations in water quality may enable aquaculture managers to implement timely interventions, such as optimizing feed usage or adjusting species stocking densities to mitigate environmental impacts.

This study is developed in the framework of an innovative inshore IMTA rearing model, performed within the EU REMEDIA Life project (LIFE16 ENV/IT/000343) in the Gulf of Taranto (Ionian Sea), where a new set of filter feeding bioremediators, such as polychaetes, sponges, 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). Previous seasonal timescale analyses on the soft and hard bottom benthic community, water column nutrient concentration, and water microbiology have demonstrated that such an IMTA system was particularly efficient at improving the environmental quality around the fish cages, through the filtering capacity of bioremediating organisms (Arduini et al., 2022; Borghese et al., 2023; Giangrande et al., 2022; Stabili et al., 2023). Among the main biomasses of the bioremediating organisms, the average annual production of $\sim 0.5 \text{ kg m}^{-1}$ of polychaetes, and $\sim 0.1 \text{ kg m}^{-1}$ of sponges was estimated (Giangrande et al., 2020). Additionally, the annual production of other invertebrates from the natural fouling community that settled on the IMTA collectors was estimated at $\sim 1.5 \text{ kg m}^{-1}$, contributing to the overall filtering capacity of the system. (Giangrande et al., 2020). In addition to the biological capture of nutrients in the water column, it is worth noting that physical sequestration of organic matter by IMTA

structures (ropes and collectors) was observed, and $\sim 0.3 \text{ kg m}^{-1} \text{ year}^{-1}$ of trapped sludge estimated to be mechanically intercepted (Giangrande et al., 2020). A short-time cycle would be a very useful complementary study for the potential changes in carbon fluxes and food availability in contrasted seasons in such IMTA facilities.

In the present study, the fluctuations in the composition of seston were investigated in the water column, by sampling every 4 h over a period of 36 h, in the aforementioned IMTA system and in a neighbouring fish facility where bioremediating organisms are absent. The observations were made in two contrasting periods of the year, one during the summer period (July 2021) and one at the end of the winter period (March 2022). These periods are considered different in many ways in temperate seas such as the Mediterranean, in terms of temperature, hydrodynamics and seston concentration/quality (Estrada, 1996). Environmental variables such as temperature, dissolved oxygen and pH were monitored, concurrently with biochemical parameters of the seston: lipids, which are among the most reliable organic components of seston in terms of food availability for filter and grazing feeders (Grémare et al., 1997; Rossi et al., 2003) and the planktonic component (chlorophyll-*a* concentration and zooplankton composition and abundance).

The main objective of this paper is to understand how seston quantity and quality may be affected by suspension feeding organism in such eutrophic conditions using a short-time cycle approach, and how such differences may potentially affect the energy fluxes in the environment impacted by the multi-trophic and traditional aquaculture systems at different times of the year.

2. Materials and methods

2.1. Study area

The study area is located on the south-west side of the Mar Grande of Taranto ($40^{\circ}25'56'' \text{ N}$; $17^{\circ}14'19'' \text{ E}$, Southeast Italy, Ionian Sea, Fig. 1). The Mar Grande of Taranto is a semi-enclosed basin reaching a maximum depth of 45 m. The local surface current is directed from the north-east to the south-west at a speed of about 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 about 1.3 cm s^{-1} .

The investigation was performed at the aquaculture plant “Maricoltura Mar Grande”, which covers a surface of 0.06 Km^2 and it's positioned at about 600 m from the coast. It consists of 15 cages ($\varnothing 22 \text{ m}$), working at a depth ranging from 7 to 12 m and producing about $100 \text{ tons} \cdot \text{year}^{-1}$ of European seabass *Dicentrarchus labrax* (Linnaeus, 1758) and 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.

Part of the plant was converted into an IMTA system by the REMEDIA life project; in this way two distinct areas of the plant are present: a control area (control site) and an area where the IMTA system is present (IMTA site; Fig. 2).

The IMTA rearing 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) Kützinger, 1845 and *Gracilaria bursapastoris* (S.G.Gmelin) P.C.Silva, 1952, is described in Giangrande et al. (2020).

2.2. Sampling design

Seston composition and physical-chemical variables were monitored every 4 h over a period of 36 h in the IMTA and in the control (Cn) sites, at two different depths: 1 m from the sea surface (s) and 1 m from the bottom (d), in the summer (July 2021) and in the winter (March 2022) periods (Table 1). In both cases, the experimental measurements started at 9 AM and ended at 5 PM the following day, for a total of 9 sampling

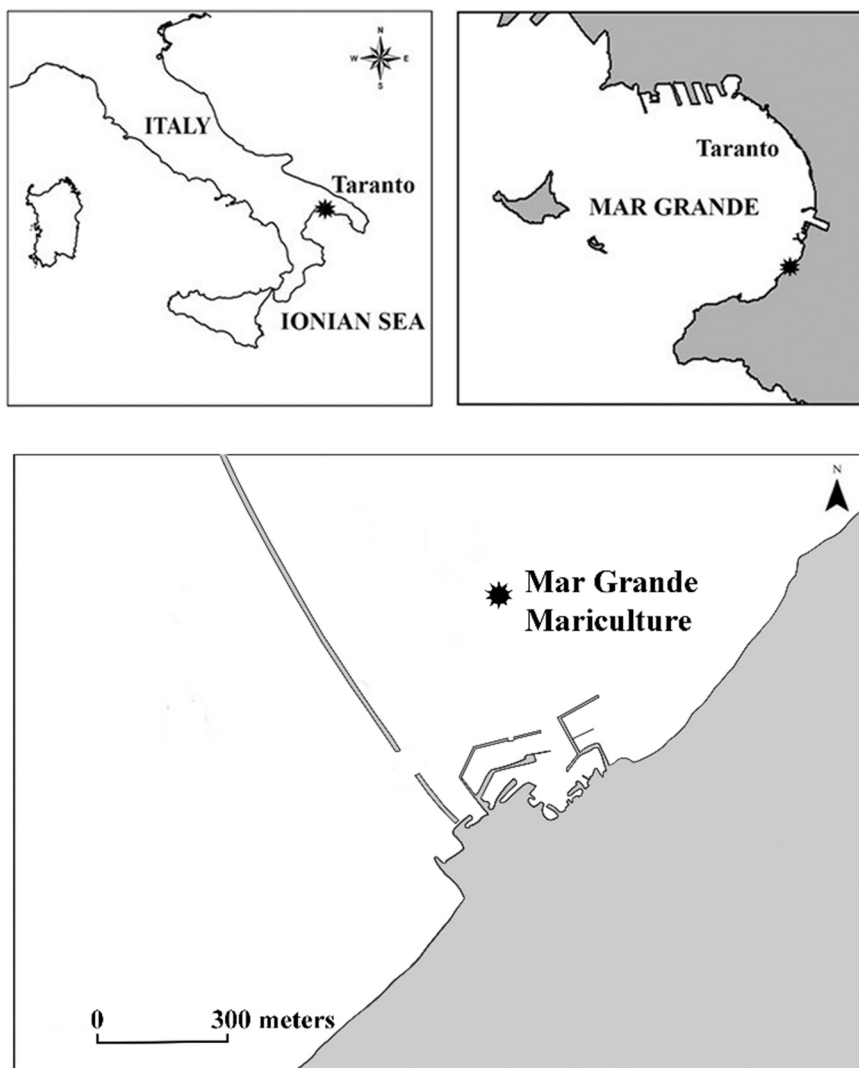


Fig. 1. Study area. *: Mar Grande Mariculture plant.

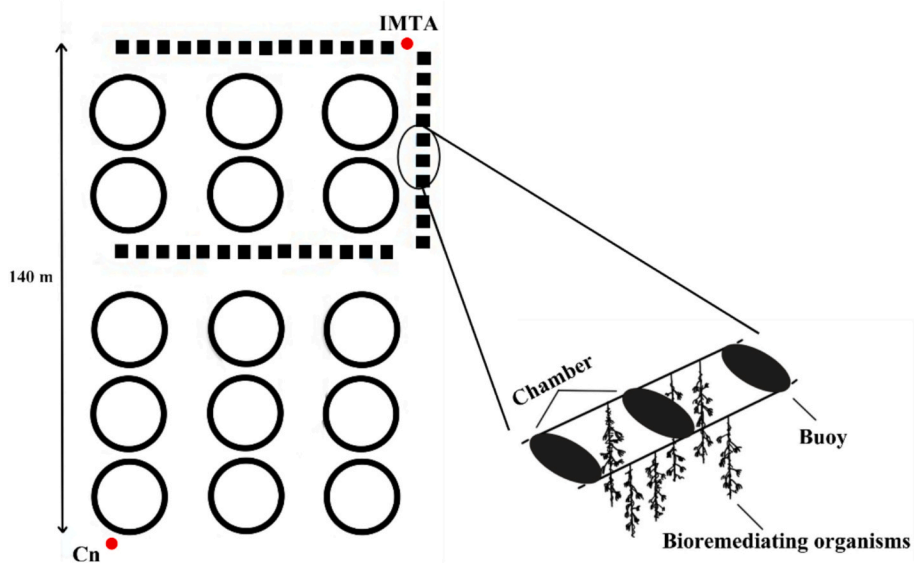


Fig. 2. Mar Grande Mariculture plant scheme. Red dots: sampling sites. IMTA site on the upper right, control (Cn) site on the lower left. Black squares: arrangement of buoys where bioremediating organisms are reared. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Sampled variables and number of replicates analysed during each sampling campaign. Chl-*a* = chlorophyll-*a*; Zoop = Zooplankton.

	Environmental variables	Chl- <i>a</i> (water)	Lipids (water)	Zoop. abundance
N° of replicates	Single measurement	3	3	3
N° of stations	2	2	2	2
N° of depths	2	2	2	Whole water column

times in each period. Previous studies have suggested that intensive 6–7-h cycles may not be sufficient to capture rapid changes in seston (Rossi and Gili, 2007; Rossi and Fiorillo, 2010). For this reason, sampling was carried out every 4^h to get a more accurate picture of the situation.

2.3. Environmental variables

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

2.4. Biochemical variables

Water samples were collected with a Niskin bottle and placed into PVC containers. The samples were immediately stored in ice (6–10 °C) and in the dark until arrival at the laboratory.

To determine the chlorophyll-*a* concentration, three 200 mL replicates were filtered through glass fiber filters (GF/F) pre-combusted (450 °C, 5 h) and stored at −20 °C. Chl-*a* was extracted in 8 mL 90 % acetone in the dark at 4 °C for 24 h at each depth and moment of the cycle. 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).

For total lipids of the water column, also three 1500 mL replicates were filtered through pre-combusted (450 °C, 5 h) GF/F filters. Total lipids were analysed using Barnes and Blackstock (1973) spectrophotometrical (colorimetric) procedure, as modified by Rossi and Fiorillo (2010). Filters were extracted in chloroform-methanol (2:1 v/v). The extract was dried, using sulphuric acid and vanillin to complete the colorimetric method. Blanks were made to control the interference of filter glass fiber particles and cholesterol was used as a standard (Grémare et al., 1997).

2.5. Zooplankton abundance and composition

Water samples were collected through a plankton net (mesh size of 80 µm). Each sampling was conducted by vertical tow along the entire water column (from 2 m above the bottom up to the surface) to prevent interfering of species vertical partitioning. The volume of water filtered during each tow, estimated with a flow meter (HYDRO-BIOS Model 438,115) placed at the net mouth, ranged approximately from $0.113 \pm 0.006 \text{ m}^3$ to $0.905 \pm 0.045 \text{ m}^3$. Samples were stored in 90 % ethanol solution and the specimens were counted and identified under an inverted microscope.

2.6. Statistical analysis

The variability in chl-*a* and lipid concentrations was assessed at two different depths and several sampling points (9 in two days) within two different sampling periods (July 2020 and March 2021) in IMTA and traditional aquaculture (control) by permutational analyses of variance (PERMANOVA, Anderson, 2001). The design consisted of four factors:

Sampling Period (PE, as random factor with 2 levels), Sampling Time (TI, as a random factor with 9 levels), Depth (DE, as a fixed factor with 2 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 9999 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 different 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 (Anderson, 2008; Clarke and Gorley, 2006). Pearson's correlation matrices and p -values were calculated using STATISTICA software.

3. Results

3.1. Environmental variables: Temperature, pH and DO

Water temperature showed similar values between IMTA and Cn sites in both periods, ranging from 22.2 °C to 26.5 °C in July and from 13.5 °C to 14.9 °C in March. In the summer period, temperature differences between depths were more pronounced, while no daily changes were observed in March, with consistent values throughout all sampling times at both depths (Fig. 3).

The recorded pH values ranged from a minimum of 7.99 up to a maximum of 8.22 in July. No clear daily pattern was observed during the warm period, but the IMTA site experienced slightly higher values than the monoculture site at the same depth. The pH values measured in March ranged from 7.94 to 8.21, with values being quite similar across sites and depths (Fig. 3).

The recorded DO values did not show a clear daily trend in either period; however, greater variations were observed in July, with values ranging from 5.51 ppm to 7.60 ppm, while the corresponding range in March was from 7.98 ppm to 8.51 ppm (Fig. 3).

3.2. Chlorophyll-*a* concentration

Chlorophyll-*a* values ranged from $0.47 \mu\text{g L}^{-1}$ to $3.75 \mu\text{g L}^{-1}$ in July and from $0.31 \mu\text{g L}^{-1}$ to $10.28 \mu\text{g L}^{-1}$ in March. Higher values were generally observed between 1 PM and 5 PM (Fig. 4). Similar trends were found in both sites and sampling seasons, whereas a peak in production was observed in Cn (d) at *5 PM. Chlorophyll-*a* concentrations varied at varying of treatment and sampling times within the investigated periods (Table 2). Post-hoc pairwise tests carried out separately within each sampling period showed significant differences in chlorophyll-*a* concentration in the water surface sampled in the Cn and near the seabed (i. e., s vs. d) only at T9 in March (Table 3).

3.3. Lipid concentration

Lipid values ranged from $7.89 \mu\text{g L}^{-1}$ to $394.87 \mu\text{g L}^{-1}$ in July and from $38.37 \mu\text{g L}^{-1}$ to $219.21 \mu\text{g L}^{-1}$ in March. A daily fluctuation in the lipid values was observed in July, with the highest values found during daylight hours, whereas in March the values were homogeneous throughout the sampling times (Fig. 5). Differences between sites were observed in the majority of the sampling times in both periods, with the IMTA site showing values considerably lower than Cn, except for one sampling time in July (9:00 PM), where the IMTA (s) site showed higher values than Cn (s). Lipid concentrations varied at varying of treatment and sampling times within the investigated periods (Table 2). Significant differences in lipid concentration were found among IMTA and Cn during July in T2, T3, T4, T6 and T9 at (s) and in T2, T3, T4, T5, T8 and T9 at (d) and during March in T4, T5, T6, T7 and T8 at (s) and in T2, T3, T4, T6, T7 and T8 at (d) (Table 3).



Fig. 3. Daily trend of Temperature (up); pH (middle) and dissolved oxygen concentration (down) in the IMTA and control sites in both periods. Cn = control; (s) = surface sites; (d) = deep sites; T = temperature; DO = dissolved oxygen; * = day two.

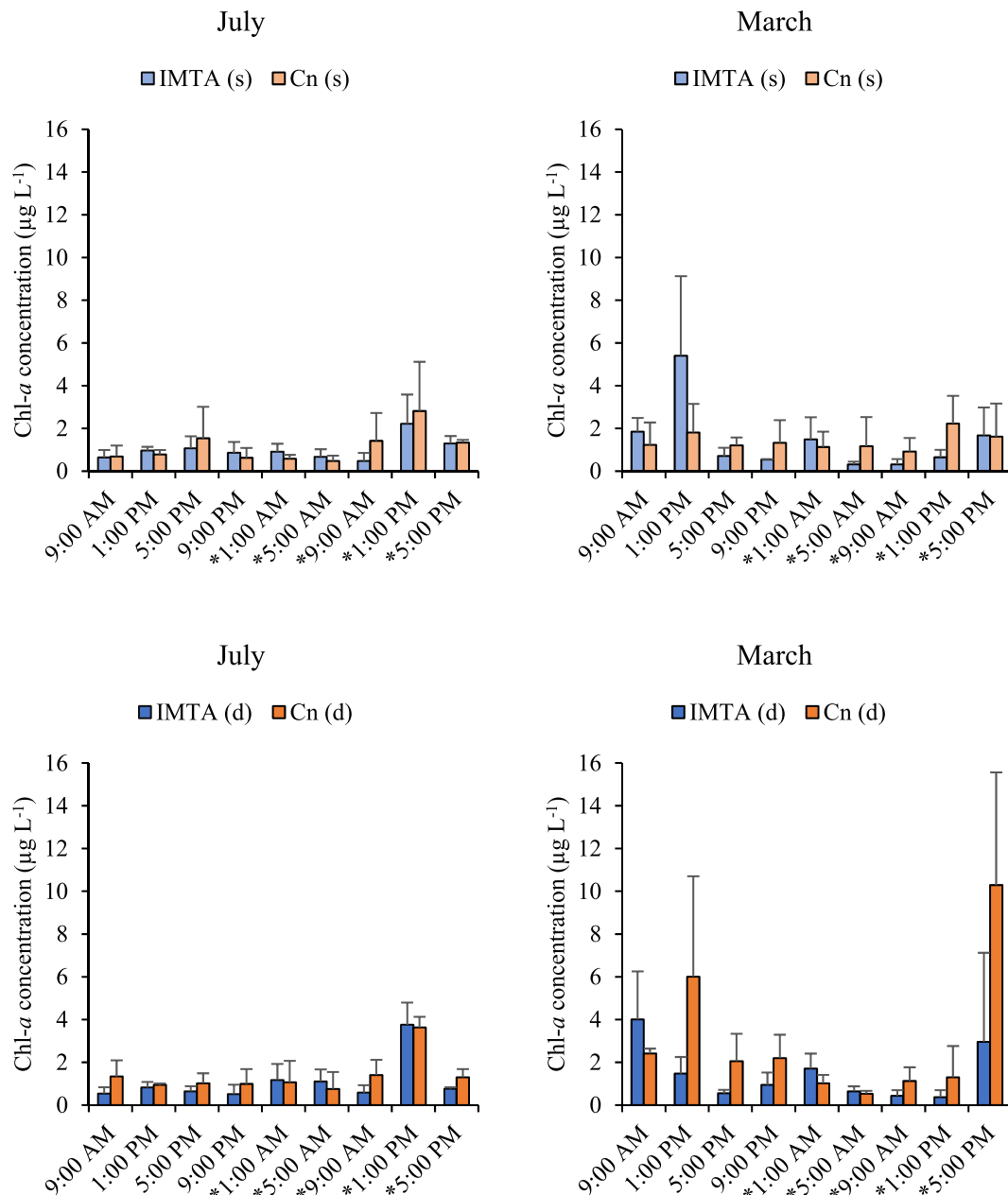


Fig. 4. Daily trend of chlorophyll-*a* in the IMTA and control sites in both periods at the surface sites (up) and deep sites (down). Cn = control; (s) = surface sites; (d) = deep sites; chl-*a* = chlorophyll-*a*; * = day two.

3.4. Zooplankton abundance and composition

Zooplankton abundance (Fig. 6) ranged from 109,021.1 ind m⁻³ recorded in the IMTA site in July to 299.9 ind m⁻³ in the IMTA site in March. In July, the abundance values at the IMTA site were always higher than those at the monoculture site, showing a sharp peak at 1 AM. On the other hand, no daily trends were observed in March.

The percentage of taxa identified showed similar values among sites, with crustaceans being the main taxon, reaching ~80 % of the total in July and ~60 % in March (Fig. 7). Differences were thus observed between periods, with a greater abundance of tunicates in March.

3.5. Correlations

Positive correlations were observed between chl-*a* and lipids at (s) and (d) in July and negative correlation between chl-*a* and pH at (d) in

March. Positive correlation was found between lipid concentration and DO at both (s) and (d) in July and between lipids and temperature in (s) sites (Table 4).

Negative correlation was evidenced between zooplankton abundance and temperature, and between zooplankton abundance and pH during March 2021 (Table 5).

4. Discussion

The results of the physico-chemical and biochemical variables showed consistent values with respect to analyses conducted in the Mar Grande of Taranto and in other areas of the western Mediterranean influenced by mariculture activities (Belmonte et al., 2013; Giangrande et al., 2022; Soriano-González et al., 2019; Stabili et al., 2023).

In particular, the water temperature showed expected values in the respective winter and summer times, with the presence of the

Table 2

Results of PERMANOVA testing for the effects of IMTA on the chlorophyll-*a* and lipid concentration at different depths across the sampling points within two different periods. df = degree of freedom; MS = mean squares; Pseudo-*F* = *F* critic; *P*(perm) = permutational level of probability. *** = *p* < 0.001.

Source	df	Chlorophyll- <i>a</i> concentration			lipid concentration		
		MS	Pseudo- <i>F</i>	<i>P</i> (perm)	MS	Pseudo- <i>F</i>	<i>P</i> (perm)
PE	1	6.957	1.806		14.956	1.561	
DE	1	3.630	2.267		1.503	1.072	
TR	1	3.621	3.070		7.712	16.228	
TI(PE)	16	3.852	7.482		9.582	142.930	
PExDE	1	1.601	1.193		1.401	2.758	
PExTR	1	1.180	1.436		0.475	0.811	
DEXTR	1	2.941	1.486		0.017	0.215	
TI(PE)xDE	16	1.342	2.607		0.508	7.580	
TI(PE)xTR	16	0.821	1.595		0.586	8.744	
PExDEXTR	1	1.979	1.394		0.080	0.152	
TI(PE)xDEXTR	16	1.42	2.758	***	0.524	7.809	***
Res	144	0.515			0.067		
Total	215						

Table 3

Results of the pairwise tests contrasting chlorophyll-*a* and lipid concentration between the levels of factors Depth (i.e., s vs. d) and Treatment (i.e., IMTA vs. control). *P* (MC) = probability level after Monte Carlo simulations; Cn = control; (s) = surface sites; (d) = deep sites; t = pairwise tests. * = *p* < 0.05; ** = *p* < 0.01; *** = *p* < 0.001; ns = not significant.

	Chlorophyll- <i>a</i> concentration					Lipid concentration			
	July; (s) vs. (d)		March; (s) vs. (d)			July; IMTA vs. Cn		March; IMTA vs. Cn	
	t	<i>P</i> (MC)	t	<i>P</i> (MC)		t	<i>P</i> (MC)	t	<i>P</i> (MC)
T1_IMTA	0.414	ns	1.596	ns	T1_s	13.851	ns	19.519	ns
T1_Cn	1.219	ns	1.910	ns	T1_d	0.329	ns	0.035	ns
T2_IMTA	0.798	ns	1.793	ns	T2_s	48.038	**	0.128	ns
T2_Cn	1.309	ns	1.486	ns	T2_d	45.227	**	4.191	*
T3_IMTA	1.257	ns	0.663	ns	T3_s	67.729	**	14.458	ns
T3_Cn	0.588	ns	1.095	ns	T3_d	4.062	*	61.692	**
T4_IMTA	0.885	ns	1.184	ns	T4_s	71.258	**	78.695	**
T4_Cn	0.746	ns	0.984	ns	T4_d	27.456	*	60.179	**
T5_IMTA	0.521	ns	0.296	ns	T5_s	22.375	ns	39.256	*
T5_Cn	0.819	ns	0.245	ns	T5_d	43.759	*	20.819	ns
T6_IMTA	1.118	ns	2.108	ns	T6_s	39.412	*	55.288	**
T6_Cn	0.567	ns	0.816	ns	T6_d	10.211	ns	28.201	*
T7_IMTA	0.352	ns	0.552	ns	T7_s	0.970	ns	30.822	*
T7_Cn	0.017	ns	0.390	ns	T7_d	13.416	ns	60.474	**
T8_IMTA	1.551	ns	1.008	ns	T8_s	24.076	ns	3.020	*
T8_Cn	0.599	ns	0.827	ns	T8_d	39.304	*	11.000	***
T9_IMTA	2.594	ns	0.506	ns	T9_s	60.104	**	1.950	ns
T9_Cn	0.193	ns	2.734	*	T9_d	67.424	**	27,748.000	*

thermocline in July, showing only a small variation in amplitude during the nighttime, as the water temperature at (d) sites rose slightly. It has been shown that the oscillation of the thermocline in coastal waters may reach >25–30 m depth in open areas (from 14 to 23 °C in few days; Cebrian et al., 1996; Rossi et al., 2011), potentially altering the metabolism of the water column organisms in summer, but especially the benthic species. In this case, the confined and shallow waters do not exhibit the same significant thermocline oscillations observed in open coastal areas, but there is still a detectable trend, that may impact the metabolic activity of sessile organisms, particularly suspension feeders. (Coma, 2002). In winter, on the other hand, the temperature remained constant, showing homogeneous values throughout the water column. This makes the metabolic demand more stable, especially in terms of oxygen consumption by the IMTA suspension feeders (Prevati et al., 2010).

Huge changes in pH daily variation were recorded, however, especially in summer in the Cn (s), but also in winter Cn (s). Our findings showed that IMTA (s) and (d) in July and IMTA (d) in March nearly always showed higher pH values than the respective depths in the Cn. Little attention has been paid to pH in studies examining the impacts of mariculture (Sarà, 2007), although the pH is a key chemical water indicator (Howland et al., 2000; Lin and Randall, 1990). A slight tendency toward acidification of impacted waters due to biological activity of

reared organisms has been observed globally, especially in more confined and shallow environments (Sarà, 2007). The observed peaks seem to consolidate this hypothesis, since the influence of the farmed fish in the Cn may have affected directly or indirectly the pH. The pH values, however, are more dependent on intrinsic hydrodynamic features of the water bodies (depth, water movements, etc., Sarà, 2007). Nevertheless, further studies on pH dynamics are needed to understand whether the presence of bioremediating organisms, including photosynthetic organisms (i.e., macroalgae), can mitigate possible causes of water acidification in IMTA systems.

Regarding the DO concentration, a decrease in DO in the water column around fish cages has been documented in several studies (Bosma and Verdegem, 2011; Cao et al., 2007; Kalantzi and Karakassis, 2006; Wu, 1995). Although the current literature could be insufficient to address properly the relationship between aquaculture effects and DO depletion in the surroundings waters (Sarà, 2007), our results shed light on the importance of such episodes, observed through the intensive sampling. The DO is one of the limiting factors for aquatic physiological metabolism, and its range is an indicator of aquatic growth conditions and pollution status (Snieszko, 1974). Our results showed that in March there was no daily variation in DO values and no difference between sites at all depths, while in July there was a more complex scenario. In particular, a difference was observed between surface and deep-water

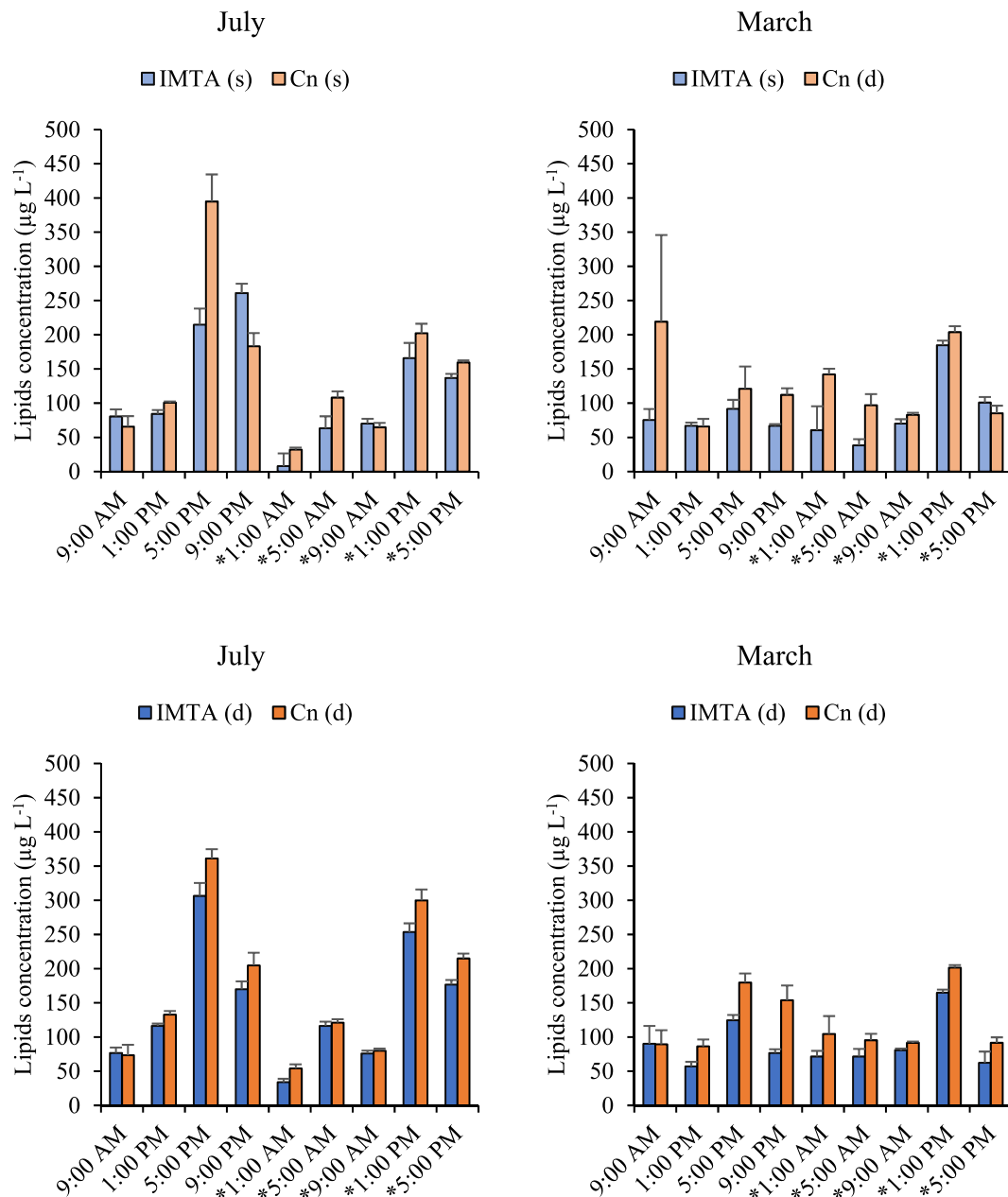


Fig. 5. Daily trend of lipids concentration in the IMTA and control sites in both periods at the surface sites (up) and deep sites (down). Cn = control; (s) = surface sites; (d) = deep sites; * = day two.

masses, with (s) sites characterized by lower DO values. Interestingly, IMTA site was characterized by higher DO values than Cn at the same depth, for the majority of sampling times. Changes in the water masses due to currents or wave action may have occurred in open waters (Rossi and Gili, 2007), but in this case the water movement is quite constant with a slow motion of particles due to slow currents (i.e., 1.5 cm s⁻¹). Again, the excess of organic matter at certain times could be responsible of such drop in DO, especially in the Cn (d), a factor that is more difficult to detect with the seasonal approach. Although more studies are needed to understand the influence of aquaculture on different environmental variables, these observations of pH and DO values of the water column in different facilities of the same area (IMTA and classic fish monoculture) may be useful in understanding the influence of bioremediating organisms, rather than that of fish farming on the surrounding environment. Positive correlation between pH and DO were found at different depths in July and March. Variations in pH and DO are

influenced by factors such as algal photosynthesis, aquatic respiration, water temperature, and the oxidative decomposition of organic matter (Scholz, 2015). Additionally, the consumption or production of carbon dioxide is typically linked to the corresponding production or consumption of oxygen. However Zang et al. (2011) suggested that these relationships in aquaculture waters, require further research to determine whether they can effectively indicate the impact of fish activity and feeding. The bioremediating organisms in the IMTA facility may thus have an effect, only detectable through the short-time cycle analysis.

Regarding the biochemical variables, chl-*a* values were consistent with a mesotrophic-eutrophic area impacted by human activity (Simbora et al., 2005; Zhang et al., 2020). No significant differences between sites were observed and the daily trend showed higher values around 1 PM and 5 PM, when there was more sun exposure. In other short-time cycle studies, fluctuation in chl-*a* may be due to sudden water

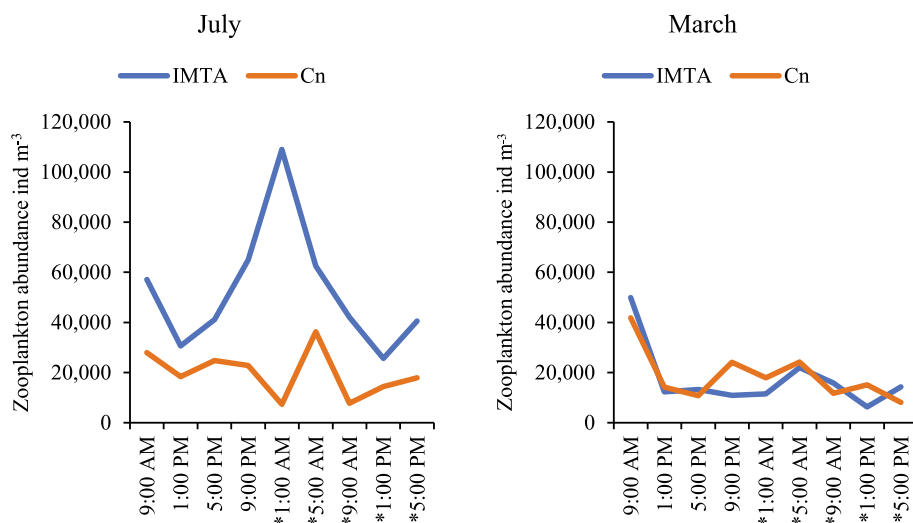


Fig. 6. Daily trend of zooplankton abundance in the IMTA and control sites in both periods. Cn = control; * = day two.

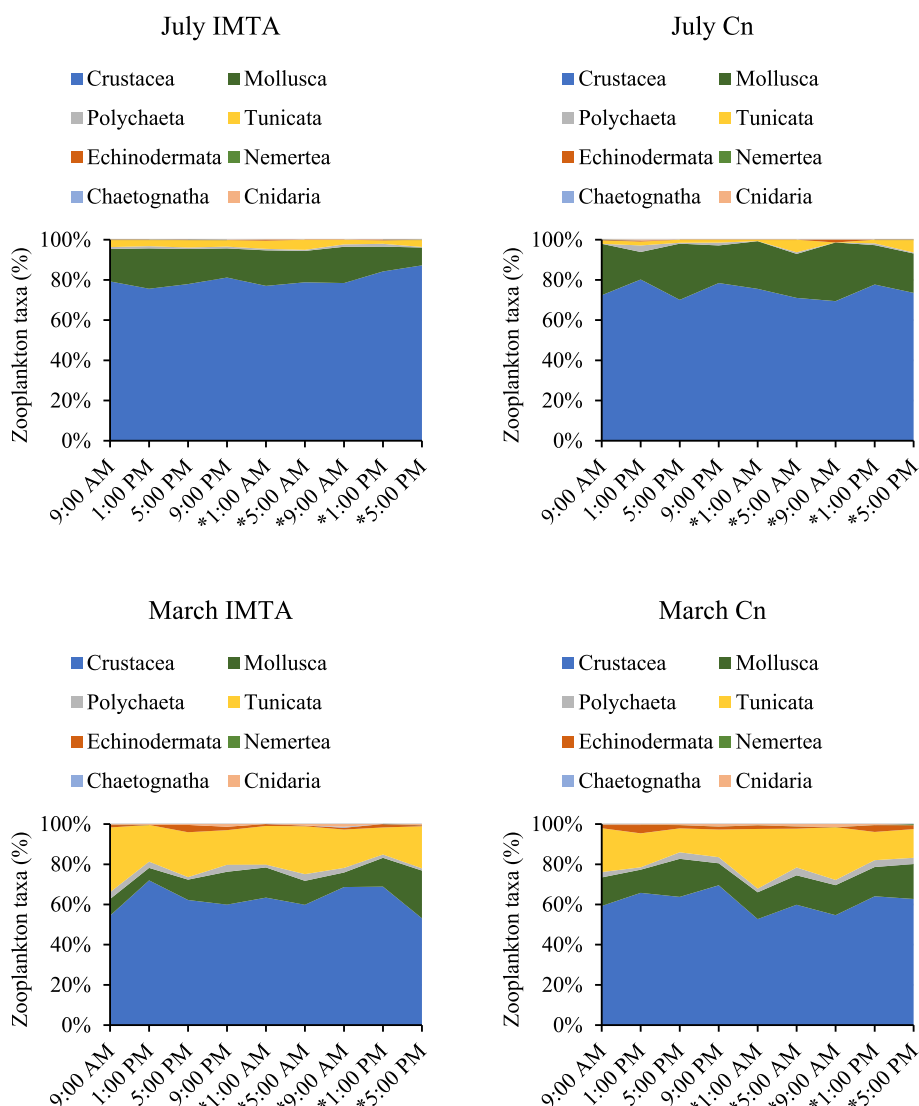


Fig. 7. Daily trend of zooplankton taxa percentage in the IMTA and control sites in July (up) and March (down) periods. Cn = control; * = day two.

Table 4

Pearson's correlation matrix for chlorophyll-*a* and lipid concentration and related environmental variables (Temperature, dissolved oxygen, pH) at (s) and (d) sites within two investigated periods (July and March). Number of samples = 54. $p < 0.05^*$. (s) = surface sites; (d) = deep sites; T = temperature; DO = dissolved oxygen; Chl-*a* = chlorophyll-*a*.

	July				(d)			
	(s)				(d)			
	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)
DO (ppm)	-0.12				*-0.59			
pH	0.09	0.18			*-0.59	*0.82		
Lipids (µg L ⁻¹)	*0.29	*0.47	-0.09		-0.01	*0.28	0.25	
Chl- <i>a</i> (µg L ⁻¹)	*0.32	-0.03	-0.03	*0.27	0.11	-0.07	-0.22	*0.34

	March				(d)			
	(s)				(d)			
	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)
DO (ppm)	0.01				0.05			
pH	0.04	*0.35			0.18	-0.14		
Lipids (µg L ⁻¹)	-0.06	-0.27	-0.11		-0.05	-0.1	0.05	
Chl- <i>a</i> (µg L ⁻¹)	0.04	0.21	-0.19	0.02	-0.08	-0.21	*-0.37	-0.07

Table 5

Pearson's correlation matrix for zooplankton abundance and related environmental variables (Temperature, dissolved oxygen, pH, chlorophyll-*a*, lipids) within two different periods (July and March). Number of samples = 27. $p < 0.05^*$. Zoop abund = zooplankton abundance; ind = individuals; DO = dissolved oxygen.

	July					March				
	Zoop abund (ind m ⁻³)	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)	Zoop abund (ind m ⁻³)	T (°C)	DO (ppm)	pH	Lipids (µg L ⁻¹)
T (°C)	0.22					*-0.48				
DO (ppm)	-0.08	*-0.59				0.06	0.05			
pH	-0.11	*-0.59	*0.82			*-0.47	0.18	-0.14		
Lipids (µg L ⁻¹)	-0.26	0.00	0.30	0.28		-0.23	-0.01	-0.02	0.10	
Chl- <i>a</i> (µg L ⁻¹)	-0.19	0.09	0.05	-0.08	*0.49	-0.14	0.22	-0.32	-0.15	-0.12

mass exchanges (Rossi and Gili, 2007), but in such area, where low currents are prevalent, the chl-*a* changes may be associated with the activity and productivity of microalgae. Thus, the quick response of the phytoplankton here observed could be linked to favourable light conditions. This is in line with an algal bloom found in mesotrophic-eutrophic waters, where the phytoplankton bloomed in few hours due to favourable conditions (Rossi and Fiorillo, 2010). These results showed up to three times more lipids and cells per litre and almost double the concentration of chl-*a* within a very short period (Rossi and Fiorillo, 2010). A positive correlation between chl-*a* and lipid concentration was found at both depths in July in the present study, also in line with previous findings linking the chl-*a* (and phytoplankton abundance) to the seston lipid concentration in an ongoing bloom area (Rossi and Fiorillo, 2010). The relationship of phytoplankton abundance and food quality (total lipids) is a factor that has to be considered, as it may be beneficial for suspension feeding organisms (i.e., bioremediators) placed in the IMTA facility.

It is accepted that high lipid contents in the seston of near-bottom water layers may be a good indicator of food availability for benthic suspension feeders (Grémare et al., 1997; Rossi et al., 2013). In the present study, concerning the lipid content of the water column, there was a clear depletion of lipids from the surrounding seston in the IMTA site, especially in March. Indeed, significant differences between sites were observed in both periods at both depths in most sampling times (Table 3). The lipidic content can be considered an indicator of a high amount of available food for benthic suspension feeders, which in this shallow coastal system could be the major controller of the phytoplankton cell concentration (Cloern, 1982). In the present study, the presence of suspension feeders could explain the lower quantity of lipids in the IMTA site, where the active filtering is depleting part of the available organic matter. This interesting finding reinforces the control

of benthic suspension feeders in near bottom seston dynamics (Rossi and Gili, 2009).

The present results also showed differences in trends between the two periods: in July there was a wider daily fluctuation than in March (i.e., DO and lipid concentration; zooplankton abundance), with higher values during daylight hours. Probably in summer, with less water movement and the larger amount of food given to fish, the trend more closely reflected the impact of aquaculture, as the highest lipid values were measured during the daytime (about twice the values recorded in March), when there was still some of the spilled feed in the water column before it was washed away by the current or accumulated in the sediment below the cages. In late spring-early summer, such fluctuations are common, and it has been shown that affect the activity of passive suspension feeders (Rossi and Gili, 2007; Rossi and Rizzo, 2021). Thus, it is likely that in this case the period of summertime makes the difference in terms of short-term fluctuations that may affect the food availability for suspension feeding organisms. The IMTA and Cn sites exhibited this same trend, demonstrating that the variation in water column lipids depended on common factors (i.e., spilled feed and its vertical and lateral transport or precipitation) in the study area beside the type of farming. However, the filtering capacity of the suspension feeders may be responsible for the differences in seston values between the sites in both periods. The majority of the biomass of the bioremediating organisms reared in the IMTA facility was estimated to belong to *S. spallanzanii*, with an average annual production of approximately 800 kg (REMEDIa Life project observation). This polychaete exhibits a wide trophic plasticity, feeding on both phytoplankton and organic material from the water column (Giangrande et al., 2005). Additionally, its pseudo-feces are compacted with mucus during tube construction, ensuring their complete removal from the system (Giangrande et al., 2005). Laboratory experiments have demonstrated the species high

nutrient removal efficiency (~40 %), with increased activity during warmer periods (Clapin, 1996; Giangrande et al., 2005), suggesting its potential to influence seston composition in the water column for bioremediation purposes (Giangrande et al., 2005).

Zooplankton has also a role in the seston depletion, and in the present set of observations may have also a role when IMTA and Cn areas are compared. Coastal zooplankton has sometimes unpredictable concentrations due to water masses movements and the relevance of sea floor topography (Calbet et al., 2001). In the present study, we studied the contrast between IMTA and Cn sites, making vertical zooplankton sampling as per other coastal studies (e.g., Calbet et al., 2001). In this case, the short-time cycle showed zooplankton abundances to be very different between seasons. In the summer intensive cycle, IMTA site showed higher abundance values than Cn, with a clear daily trend characterized by a peak at 1 AM. In winter, by contrast, no clear trend of abundance and no differences between sites were found, probably due to the increased current, as evidenced by the homogeneous daily trends of the other variables analysed. The concentration was clearly higher in the IMTA site in summer, being similar (around 20,000 individuals m^{-3} ; Fig. 6) in the winter IMTA and Cn sites and the Cn site in summer. These differences between the two sampling campaigns are in line with what emerged from the seasonal study of zooplankton in the same area (Borghese et al., 2025) and other previous near bottom studies in the Mediterranean sea (in terms of composition; Coma et al., 1994; Rossi et al., 2004), since the late summer-autumn period was characterized by higher numbers of individuals in the IMTA site than in the Cn, whereas in the winter period no differences were found between sites. Interestingly, the presence of active filter feeders in our study seems to be related with the abundance of zooplankton in summer. The presence of these three-dimensional living structures may indicate an effect on zooplankton in terms of available seston (particle retention; Guizien and Ghisalberti, 2015) and shelter (Frutos et al., 2017), being the lipid depletion also due to the presence of this pelagic organisms that graze over the particles. Nelson and Bramanti (2020) suggested an important role of the marine animal forest canopy in the seston concentration, including zooplankton. We suggest that the complexity gives shelter and offers a higher variety and quality of food that remains in the three-dimensional alive structures trapped and reworked by the organisms of the microbial loop.

No major differences were found in the percentage of sampled zooplankton taxa between sites, supporting the hypothesis of plankton retention by farm structures (and IMTA collectors) rather than a selective attraction by chemical cues (Fernandez-Jover et al., 2016; Klebert et al., 2013; Madin et al., 2010). Differences are instead present between the two different periods, with winter sampling characterized by a greater presence of tunicates. However, more accurate identification efforts are needed for a better assessment of the zooplankton diversity.

The present work emphasizes the importance of short-time cycles in the observation of potential seston availability and depletion if suspension feeding organisms are present. Various environmental factors, such as storms, river runoff, tidal currents, and more, can lead to sporadic disturbances within coastal biological communities. These disturbances introduce inputs that, in terms of biological production, can be quantitatively equivalent to changes seen during seasonal transitions (Alongi, 1998). Notably, in the context of seston dynamics, the impact of these factors can be equally significant when comparing daily and yearly trends (Sournia, 1975). Taylor and Howes (1994) demonstrated that the accuracy of computed seasonal production improves with more frequent sampling. These observations strongly advocate for short-timescale field sampling. Such sampling methods are crucial for revealing subtle, smaller scale changes in ecosystems that often remain unnoticed in traditional monthly sampling. They can significantly contribute to our understanding of near-bottom seston dynamics, and, consequently, the frequency of food availability for benthic communities (Rossi and Gili, 2007; Van et al., 1997).

5. Conclusions

In conclusion, significant differences were found between sites at different sampling times in lipid concentration values, suggesting a positive impact of the marine animal forests within the IMTA facilities on the seston. These findings support the effectiveness of this system in mitigating the negative impact of fish farming. The short-time cycle proved to be very useful to detect changes within hours, especially in summertime, where more fluctuations were detected due to the water stratification. In the study area (Mar Grande, Taranto), the increased seawater temperature can raise the coastal productivity (and probably nutrients, De et al., 2022), supporting a water column biomass that feeds suspension feeding organisms. Having a hard substrate (i.e., the ropes) the settled suspension feeders make a complex and biodiverse opportunistic “habitat” that helps to bioremediate the aquaculture waste.

Hundreds of vertical collectors at the IMTA site may create the framework for a real animal forest structuring (sensu, Rossi et al., 2017), capable of increasing the abundance of zooplankton, providing protection, food and preventing the drift of organisms, but also influencing near bottom seston biogeochemical cycles. This opens the possibility to think about using such canopy effect in terms of biomass and biodiversity enhancement due to the presence of these ecosystem engineering species (sensu, Jones et al., 1994) as restoration scenarios (Giangrande et al., 2021; Rossi and Rizzo, 2020). The changeover of inshore fish farms, which cause environmental pressure, to well-designed IMTA system, coupled with the creation of underwater gardens or animal forests, can lead to the restoration of the habitat as a whole (Giangrande et al., 2021), being a source of leisure and education in controlled conditions (Rossi and Rizzo, 2020).

Further studies at different temporal resolutions are needed to better understand seasonal and daily trends in other plants as well with different environmental conditions to understand whether the bioremediation pattern can be generalized to a broad spectrum of environmental conditions.

CRedit authorship contribution statement

Jacopo Borghese: Writing – original draft, Visualization, Validation, Resources, Investigation, Data curation, Conceptualization. **Adriana Giangrande:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Daniele Arduini:** Resources, Investigation. **Roberta Trani:** Resources, Investigation. **Lorenzo Doria:** Resources, Investigation. **Marco Anglano:** Resources, Investigation, Data curation. **Joseba Aguilo-Arce:** Resources, Investigation. **Andrea Toso:** Resources, Investigation. **Matteo Putignano:** Resources, Investigation. **Lucia Rizzo:** Writing – review & editing, Visualization, Validation, Formal analysis. **Sergio Rossi:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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. If there are other authors, they 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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Data availability

Data will be made available on request.

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