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# Impact on the water column biogeochemistry of a Mediterranean mussel and fish farm

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## Abstract

We investigated and compared the impact of organic loads due to the biodeposition of mussel and fish farms on the water column of a coastal area of the Tyrrhenian Sea (Western Mediterranean). Physico-chemical data (including oxygen, nutrients, DOC and particulate organic matter), microbial variables (picoplankton and picophytoplankton density and biomass) and phytoplankton biomass (as chlorophyll-*a*) were determined on a monthly basis from March 1997 to February 1998. The results of this study indicate that both fish farm and mussel culture did not alter significantly dissolved inorganic phosphorus and chlorophyll-*a* values, while inorganic nitrogen concentrations. In contrast, no significant differences were observed comparing particulate matter concentrations. The increased DOC concentrations determined a response of the heterotrophic fraction of picoplankton, while picophytoplankton, likewise phytoplankton, did not display differences among fish or mussel farms and control site. From the analysis of the heterotrophic components, it is possible to conclude that the impact of fish farms is evident only for the heterotrophic components. The comparative analysis of the mussel biodeposition and fish-farm impact revealed that mussel farms induced a considerably lower disturbance, apparently limited to an increased density and biomass of microbial assemblages beneath the mussel cultures. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Aquaculture impact; Picoplankton; Dissolved organic carbon; Mediterranean Sea; Mussel farm; Fish farm

## 1. Introduction

The rapid expansion of aquaculture activities, particularly fish and mussel farming, in coastal areas is generating an increasing concern over their environmental impact [1,2]. The impact of mariculture is due to the increased nutrient loads, particularly organic phosphorus and nitrogen and inorganic nitrogen (ammonia) that might easily induce eutrophication [3]. Fish farming and mussel cultures are expected to have a completely different impact over the environment; fish farms produce a net input of nutrients, whereas mussel cultures remove particulate organic matter (POM) from the water column, but also increase sedimentation rates by producing faecal and pseudo-faecal material [4,5].

Due to the high biodeposition, both fish and mussel farms create farm sediments that are characterised by suboxic to anoxic conditions [1,2,6]. Often, biodeposition processes affect benthic community structure [1,2,7,8,9], altering environmental conditions in a wider bottom area [10].

An over enrichment of organic carbon, filrogen and phosphorus in the water column could lead to extensive

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eutrophication [5], with increased oxygen consumption due to the increased organic matter supply [11]. Biodeposits stimulate benthic microbial biomass and productivity [12], playing a fundamental role on organic matter turnover and related biogeochemical cycles [13].

Aquaculture activities are also expected to provide a higher impact on the sediments than on the overlaying waters [14]. Beveridge [15] reviewing a large number of studies, concluded that in marine waters the enhanced nutrient concentrations did not affect phytoplankton growth, and similar results were reported by Pitta et al. [16] from the analysis of microzooplankton assemblage structure. Information on the impact of aquaculture activities over the water column is very scant [15,16], and, to our knowledge, the bacterioplankton response to fish and mussel farm impact is practically non-existent. In oligotrophic areas, as the Mediterranean Sea, the pelagic microbial loop is expected to represent a sensitive descriptor of changes in trophic conditions, and picoplankton assemblages (i.e., microorganisms smaller than 2 µm), due to their very high turnover rates and their role in biogeochemical cycles, are assumed to respond rapidly to any environmental change [17].

This study was designed to investigate the effects of farm activities on the water column in coastal area of the Western Mediterranean. Physico-chemical parameters (including dissolved organic carbon (DOC), POM, and nutrients), phytoplankton biomass (as chlorophyll-*a*) and microbial variables (including the abundance, biomass and structure of picoplankton assemblages) were investigated in order to: (a) identify changes in water column trophic state; (b) investigate consequences on microbial assemblages; (c) compare the impact of fish and mussel farms.

#### 2. Materials and methods

#### 2.1. Study area

Sampling was carried out once each month from March 1997 to February 1998 in the Gaeta Gulf (Tyrrhenian Sea, NW–Mediterranean Sea;  $41^{\circ}21'$  N;  $13^{\circ}60'$  E; Fig. 1). The area is characterised by microtidal regime (about 30 cm) and the presence of two river estuaries. Dominant currents flow in SE–NW direction following the cyclonic circulation of the Tyrrhenian Sea. The study area is sheltered and characterised by the presence of sandy–muddy sediments. *Posidonia oceanica* meadows are present in the northern part of the study area, although of limited extent. Visibility was highly reduced in the entire study area independently from the presence of intensive fish farming ( $250 \text{ tyr}^{-1}$  of *Dicentrarchus labrax* and *Sparus aurata*,  $18 \text{ kg m}^{-3}$  final



Fig. 1. The sampling stations in the Gaeta Gulf, Tyrrhenian Sea (Western Mediterranean). The shadow box represents the mussel farm area and the circular box represents the fish farming area.

biomass) and bivalve mollusc cultivation activities  $(400 \text{ t yr}^{-1})$ .

## 2.2. Sampling strategy

In order to estimate the impact due to mussel and fish farms, after a preliminary survey based on a grid of stations over a wide sector of the Gulf, three sampling stations were selected. One station was located inside of the Mussel-farm area (hereafter reported as the Mussel station), one was located inside the Fish-farm area (hereafter reported as the Cage station) and a third station (Control) was located at about 1 km far from the cage and mussel cultivation, in a north–northeast area not affected by the aquaculture plants. The three stations were located on the 12m isobath. Seawater samples were collected at all sampling stations on monthly basis, from March 1997 to February 1998, using Niskin bottles at surface (-1 m) and at 10m depth.

## 2.3. Environmental variables

Temperature (°C) and oxygen  $(mg]^{-1}$  and % of saturation) were measured using a multiparametric probe (Hydrolab, Inc. Austin, USA). Chlorophyll-*a* (Chl-*a*;  $\mu g l^{-1}$ ) was determined according to Lorenzen and Jeffrey [18]. Chlorophyll-*a* concentrations were converted to carbon content (C-Chl-*a*) using a conversion factor of  $30 \mu g C \mu g Chl^{-1}$ . Dissolved nutrient concentrations were determined according to Strickland and Parsons [19]. Data on total inorganic nitrogen (DIN) and inorganic phosphorus (DIP) were reported as the average of the two sampling depths. The analysis

of dissolved organic carbon (DOC;  $mg1^{-1}$ ) was carried out on Whatman GF/F filtered water samples after acidification (i.e., as the difference between the total and inorganic dissolved carbon), by means of a Shimadzu TOC Analyzer (Inc. Japan; Mod. 5050). POM composition and carbon content (as biopolymeric carbon concentrations BPC;  $\mu gC1^{-1}$ ) were determined according to Fabiano et al. [20].

# 2.4. Microbial variables

Water samples (500 ml) were immediately prefiltered, under gentle vacuum (<50 mm Hg), onto 47 mm diameter 2 µm pore-size Nuclepore filters to separate picoplankton (0.2-2 µm) from larger cells and to prevent filter clogging and then fixed with 0.2 µm filtered formaldehyde (2% sample final concentration). Total picoplankton and picophytoplankton were estimated using epifluorescence microscopy according to Maugeri et al. [21]. Briefly, for the determination of picoplankton  $(0.2-2 \,\mu\text{m})$  three replicates were filtered onto 25 mm diameter 0.2 µm black Nuclepore filters. Total picoplankton counts were obtained by staining cells with DAPI (4'6-diamidino-2-phenylindole). Picoplankton cells were counted using epifluorescence microscopy on at least 10 fields randomly selected for a total count of more than 400 cells. Picoplankton biovolume estimated by means of cell shape after measurement with a micrometric ocular and was converted into biomass  $(\mu gCl^{-1})$  assuming 310 fgC  $\mu m^{-3}$  [22]. Picophytoplankton cells were classified as either prokaryotes (cyanobacteria) or eukaryotes according to their autofluorescent spectrum: phycoerythrin-phycocyanin rich cyanobacteria (yellow-orange fluorescence) and chlorophyll-dominant (red-green fluorescence), respectively. Cyanobacteria densities were converted into biomass ( $\mu$ gCl<sup>-1</sup>) assuming 294 fgC cell<sup>-1</sup> [23]. The biovolume of eukaryotic cells was also estimated by means of micrometric ocular and converted into biomass using a conversion factor of  $220 \, \text{fgC} \, \mu \text{m}^{-3}$ [24]. The total picophytoplankton biomass was determined as the sum of cyanobacteria biomass and eukaryotic biomass.

## 3. Results

## 3.1. Environmental variables

Data relative to temperature and oxygen are reported in Table 1. Temperature ranged from 12.7 to 25.7°C did not display differences among sampling sites. Oxygen values were close to saturation at surface during the entire sampling period. Lowest saturation conditions were observed at 10 m depth in the mussel and fish-farm areas.

	Temnerg	(Jo) entit					Ovvian (n	1-1)					Ovvicen o	aturation (6	176			
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	Control		Mussel		Cage		Control		Mussel		Cage		Control		Mussel		Cage	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Mar	13.9	14.9	13.7	13.8	13.9	13.8	7.7	7.5	7.4	7.2	7.4	7.5	93.3	91.1	89.2	87.7	90.4	91.2
Apr	17.0	17.2	17.0	16.7	17.1	16.7	7.6	7.3	7.4	7.2	7.5	7.1	97.9	94.5	96.1	92.6	97.7	92.5
May	20.1	19.6	20.4	19.6	20.4	19.7	7.5	7.1	7.5	7.1	7.5	6.8	102.4	97.8	103.0	97.5	105.0	93.7
Jun	17.9	16.5	18.4	16.6	19.5	16.7	7.5	7.8	7.1	7.8	7.2	7.8	100.0	101.0	95.8	103.6	99.5	99.5
Jul	24.6	20.3	24.8	22.5	24.6	20.5	7.0	7.6	6.8	7.2	6.7	6.7	106.5	105.9	103.3	105.2	99.7	95.3
Aug	25.7	25.3	25.6	25.2	25.7	24.9	6.7	6.3	6.5	5.2	6.5	5.9	103.5	98.1	100.6	80.0	102.3	89.9
Sep	22.3	23.2	22.9	23.1	22.6	23.0	6.6	6.5	6.5	6.0	6.7	6.6	95.3	96.1	94.9	88.2	97.7	96.7
Nov	15.4	18.3	15.3	18.3	15.4	18.3	T.T	6.8	7.6	6.7	7.6	6.9	94.5	90.8	93.4	89.6	93.8	93.1
Dec	13.5	• 15.6	13.3	15.7	13.6	15.6	8.0	7.2	8.0	6.9	8.0	7.1	94.2	91.1	93.4	88.0	94.4	90.6
Jan	12.7	14.0	13.0	13.9	13.7	13.8	7.9	7.7	7.9	7.8	7.8	7.8	94.2	94.6	94.3	94.8	95.7	95.0
FebO	13.1	14.8	13.2	14.8	12.6	14.8	9.4	7.7	9.6	7.7	9.8	7.9	110.0	95.6	112.9	95.7	115.2	98.3
Vavg	17.8	18.2	18.0	18.2	18.1	18.0	7.6	7.2	7.5	7.0	7.5	7.1	99.3	96.1	97.9	93.0	99.2	94.2
H SEa	4	1-1	4.1	1.2	1.4	1.1	0.2	0.1	0.3	0.2	0.3	0.2	1.7	1.4	2.0	2.2	2.0	0.9
\$ 	× 50																	

Data on dissolved inorganic nutrient concentrations are reported in Table 2. DIN values at the control station were significantly lower than at the Mussel stations (paired *t*-test: p < 0.05), while DIP values did not display significant differences among sampling stations.

Chlorophyll-*a* concentrations, dissolved organic carbon (DOC) and biopolymeric carbon (BPC) are reported in Table 3. At all stations the highest total chlorophyll-*a* values were found in the surface layer  $(1.4\pm0.3\,\mu\text{g}\,\text{l}^{-1}$  at Control and  $1.3\pm0.3\,\mu\text{g}\,\text{l}^{-1}$  at the Mussel and Cage station, respectively), than bottom layer  $(0.8\pm0.2\,\mu\text{g}\,\text{l}^{-1}$  at Control,  $0.8\pm0.1\,\mu\text{g}\,\text{l}^{-1}$  at the Mussel and  $0.9\pm0.1\,\mu\text{g}\,\text{l}^{-1}$  at Cage station) but no significant differences were found among stations.

Annual mean values of DOC were  $8.1\pm1.9$ and  $9.5\pm2.4 \text{ mgl}^{-1}$  at surface and bottom waters of Control station,  $10.2\pm1.9$  and  $9.3\pm1.7 \text{ mgl}^{-1}$  at surface and bottom waters of the Mussel station and  $14.1\pm4.2$  and  $11.4\pm3.4 \text{ mgl}^{-1}$  at the Cage station. Temporal trends of DOC concentrations in Control, Mussel and Cage stations are showed in Fig. 2. Significant differences were observed comparing DOC concentrations at the Cage and Control station (paired *t*-test: p < 0.05).

Annual mean of BPC concentrations were  $153.1 \pm 29.5$  and  $94.1 \pm 20.8 \,\mu g \, l^{-1}$  at surface and bottom waters of the Control station,  $136.8 \pm 26.7$  and  $82.5 \pm 18.0 \,\mu g \, l^{-1}$  at surface and bottom waters of the Mussel station and  $144.3 \pm 29.4$  and  $96.4 \pm 23.0 \,\mu g \, l^{-1}$  at surface and bottom waters of the Cage station. At all stations, highest BPC concentrations were found in the surface layer, but BPC concentration did not change significantly among sampling sites.

## 3.2. Picoplankton assemblages

Data on total picoplankton and picophytoplankton are reported in Tables 4 and 5, respectively. During the entire study period, total picoplankton abundance at all stations displayed higher (about double) densities at surface. Annual average densities were  $10.26 \pm 0.75$  and  $5.69 \pm 0.11 \times 10^8$  cells l<sup>-1</sup>, respectively, in surface and bottom waters at the Control station. At the Mussel station, picoplankton abundance was on average,  $10.75 \pm 0.61$  and  $6.47 \pm 0.08 \times 10^8$  cells l<sup>-1</sup>, respectively, in surface and bottom waters. At the Cage station total picoplankton density was on annual average  $13.39 \pm 0.57$  and  $6.11 \pm 0.11 \times 10^8$  cells  $1^{-1}$ , respectively, in surface and bottom waters. Picoplankton density at the Cage station were significantly higher than at the Control (paired *t*-test: p < 0.05). Similar patterns were reported for picoplankton biomass.

Picophytoplankton densities were one order of magnitude lower than total picoplankton densities, but reflected the same temporal patterns. Picophytoplankton abundance ranged from 0.33 to  $4.82 \times 10^7$  cells  $1^{-1}$ . at the Control station; from 0.48 to  $4.97 \times 10^7$  cells l<sup>-1</sup>, at the Mussel station and from 0.29 to  $5.38 \times 10^7$  cells l<sup>-1</sup>, at the Cage station. No significant differences were observed among sampling sites. The prokaryotic cells (cyanobacteria) outnumbered eukaryotes on average by nearly two order of magnitude at all stations. The mean percentage contribution of eukarvotic cells to the total picophytoplankton was 1.6% and 1.4% (at 0 and 10m depth, respectively) at the Control station, 2.0% and 4.6% (at 0 and 10m depth, respectively) at Mussel station and 4.1% and 3.3% (at 0 and 10 m depth, respectively) at Cage station.

Table 2

Temporal variations of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) concentrations. Reported are values integrated along the water column and expressed as  $\mu M$ 

	DIN (µM)			DIP (µM)		
	Control	Mussel	Cage	Control	Mussel	Cage
Mar	0.27	0.39	0.27	nd	0.39	nd
Apr	0.45	0.76	0.47	0.05	0.04	0.04
May	0.26	0.80	0.33	nd	nd	0.02
Jun	0.39	0.27	0.21	0.01	0.01	0.02
Jul	0.88	0.33	0.26	nd	0.17	0.05
Aug	0.32	2.45	1.73	nd	nd	nd
Sep	0.29	0.30	0.25	nd	0.36	0.36
Nov	nd	0.47	nd	0.01	0.07	0.01
Dec	1.80	3.79	1.63	nd	nd	nd
Jan	nd	0.98	0.72	0.10	0.07	10010
Feb	0.60	1.62	0.80	0.08	0.06	0.99
avg	0.59	1.11	0.67	0.05	0.15	LE 60.31
$\pm \tilde{S}E^{a}$	0.15	0.34	0.17	0.01	0.04	0 <sup>M</sup> 0.13

<sup>a</sup>SE = Standard Error.

	Chloropl	hyll-a (µgl <sup>-</sup>	1) (1				DOC (mg	l <sup>-1</sup> )					Biopolyn	neric carbor	ι (µgC 1 <sup>-1</sup> )			
	Control		Mussel		Cage		Control		Mussel		Cage		Control		Mussel		Cage	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Mar	0.1	0.6	0.8	0.7	0.9	0.6	9.2	4.7	14.4	6.9	4.3	3.7	303.0	183.1	169.9	113.5	294.8	150.1
Apr	1.1	0.7	0.3	0.5	1.0	0.7	2.5	3.0	15.4	8.4	9.9	0.8	81.9	69.0	69.69	40.1	175.0	48.5
May	1.7	1.0	3.4	1.5	1.1	1.4	21.5	22.7	16.0	16.3	43.3	21.4	122.0	45.9	181.4	22.3	163.3	32.8
Jun	0.8	0.0	0.2	0.0	0.5	0.1	8.8	7.9	20.7	5.5	18.5	13.4	72.9	64.1	87.1	43.1	63.8	43.7
Jul	0.5	0.7	0.4	0.7	0.8	0.6	3.2	8.1	1.2	10.3	2.8	2.2	30.7	45.5	47.3	42.7	47.0	65.7
Aug	0.6	0.6	0.5	0.8	0.6	1.1	15.0	26.0	8.6	11.7	37.3	36.5	203.2	72.7	47.1	90.0	63.0	123.2
Sep	0.8	0.8	0.6	1.0	0.8	0.9	12.7	6.6	2.9	1.8	1.6	1.6	77.0	70.9	92.4	41.1	53.9	67.3
Nov	2.0	0.5	1.6	0.4	1.5	0.8	2.9	9.7	12.7	18.6	12.4	19.5	117.3	32.3	120.5	72.0	132.0	45.6
Dec	3.2	1.7	2.9	1.5	2.6	1.0	0.9	3.0	3.8	8.1	14.5	2.4	175.3	109.0	154.8	90.3	153.4	92.3
Jan	1.6	1.8	1.7	1.4	1.5	1.2	5.5	11.7	4.9	14.3	6.0	15.3	159.5	80.8	178.5	119.5	101.0	92.6
Feb	3.2	0.7	2.3	0.8	3.5	1.1	6.5	0.8	12.0	0.8	5.0	8.5	341.3	262.3	355.8	233.4	340.4	298.7
avg	1.4	0.8	1.3	0.8	1.3	0.9	8.1	9.5	10.2	9.3	14.1	11.4	153.1	94.1	136.8	82.5	144.3	96.4
$\pm SE^{a}$	0.3	0.2	0.3	0.1	0.3	0.1	1.9	2.4	1.9	1.7	4.2	3.4	29.5	20.8	26.7	18.0	29.4	23.0

Table



Fig. 2. Temporal variations in DOC concentrations at 0 m depth (a) and 10 m depth (b), at Control, Mussel and Cage station.

Picophytoplankton biomass, on annual average, at the Control station was  $7.04\pm0.31$  and  $6.29\pm0.16\,\mu gC\,l^{-1}$ , respectively, at surface and bottom waters; at the Mussel station was  $6.82\pm0.23$  and  $6.15\pm0.15\,\mu gC\,l^{-1}$  and at the Cage station  $7.72\pm0.28$  and  $6.87\pm0.09\,\mu gC\,l^{-1}$ , respectively, at surface and bottom waters.

## 4. Discussion

SE = Standard Error.

Coastal aquaculture, and particularly fish farming, are expected to produce wastes characterised by large proportion of N and P released in solute form into the water column. However, previous studies, dealing with the analysis of plankton response to fish farming, showed no significant differences between cages and control sites [16]. In addition several studies failed to establish a relationship between farm waste and phytoplankton growth in open sea, even when large inorganic nutrient inputs were observed [15].

The results of this study indicate increased inorganic nutrient concentrations in the water column of both mussel and fish farms, when compared to the control.

	Total p	oicoplank	ton abund:	ance									Total pi	icoplani	kton bic	mass							
	Contro.	1			Mussel				Cage				Control			V	fussel			Cag	ge		
	Surface		Bottom		Surface		Bottom		Surface		Bottom		Surface	1	Bottom	s	urface	Bc	ottom	Sur	face	Bott	m
	$n \times 10^8$	$1^{-1} \pm SD^{i}$	$n \times 10^{8}  l^{-1}$	$\pm SD^{a}$	$n \times 10^{8} l^{-1}$	$\pm SD^{a}$	$n \times 10^{8}  \mathrm{l^{-1}}$	$\pm SD^{a}$	$n \times 10^{8}  \mathrm{l^{-1}}$	$\pm SD^{a}$	$n \times 10^8 \mathrm{I}^{-1}$	$\pm SD^{a}$	$\mu g C 1^{-1}$	± SD <sup>a</sup> µ	μgC1 <sup>-1</sup> :	±SD <sup>a</sup> µ	gC1 <sup>−1</sup> ±	SD <sup>a</sup> μg	Cl <sup>−1</sup> ±	SD <sup>a</sup> µgC	C1 <sup>−1</sup> ± 5	D <sup>a</sup> µgC	$[-1 \pm S]$
Mar	1070	6 3K	6 47	92.0	30.00	5 20	13 16	0.66	44.46	1 01	0 37	0.15	184.80	5 11 3	36.01	1 10	11 10	3 27 0	1 60 4	3 710	y 33	0 61 8	1 1 1
ADI	30.31	00 4.06	0.47 10.28	0.06	27.69	5.84 5.84	9.16 9.16	0.02	44.40 38.99	7.14	0.37 10.82	0.05	156.85	21.03 6	53.73 C	1.24 1.38 1.	41.11 2	9.74 5	2.45 0.	13 198	.70 36.	41 90.1.0	0.43
May	8.90	0.56	4.84	0.32	9.19	0.98	4.45	0.23	6.64	1.00	7.12	0.88	95.22	5.96 3	30.00 2	01	75.10	8.05 3	2.13 1.0	55 56	.39 8.	45 44.1	5.44
Jun	4.51	0.36	3.70	0.13	5.14	0.06	3.27	0.11	5.61	1.03	3.30	0.25	33.31	2.69	34.01 1	.20	48.91	0.59 1	1.23 0.3	39 43	.67 8.	02 15.5	1.17
Jul	6.25	0.53	8.04	0.29	7.52	0.85	7.75	0.34	7.00	1.02	8.54	1.07	49.11	4.19	51.12	.84	56.16	6.34 7	0.68 3.	3 52	.7 7.	57 39.4	4.96
Aug	5.63	0.77	8.03	0.73	6.20	0.80	12.82	0.49	8.39	0.51	7.58	0.56	27.37	3.76	34.58 3	3.16	50.18	6.45 12	6.01 4.8	87 79	.14 4.	80 33.2	2.45
Sep	4.13	0.67	3.94	0.20	4.32	0.32	4.70	0.54	3.97	1.01	5.85	0.89	31.46	5.10	22.58 1	.17	20.66	1.53 2	8.02 3.2	23 21	.17 5.	37 22.8	3.49
Nov	5.11	0.41	3.26	0.19	5.88	0.73	2.62	0.56	4.98	0.54	2.41	0.12	34.11	2.71	16.88 (	.97	49.94	6.22 1	3.32 2.8	33 41	.93 4.	57 12.6	0.61
Dec	8.02	0.76	6.86	1.22	8.60	0.35	4.99	0.07	8.74	0.74	5.50	0.42	66.79	6.30	32.78 5	5.82	71.71	2.96 2	8.59 0.4	t2 41	.07 3.	48 23.6	1.83
Jan	7.73	0.27	4.77	0.13	6.02	0.42	4.79	0.71	6.61	0.75	4.77	0.73	41.85	1.47	31.44 (	.83	38.27	2.70 2	3.67 3.5	51 43	.11 4.	86 28.4	4.37
Feb	5.21	0.29	2.41	0.34	6.72	1.20	3.47	0.15	11.86	0.98	2.96	0.44	38.10	2.15	13.78 1	.97	54.44	9.69 1	2.22 0.5	53 97	.92 8.	9.9	1.48
avg∃	±SE <sup>b</sup> 10.26	0.75	5.69	0.11	10.75	0.61	6.47	0.08	13.39	0.57	6.11	0.11	69.00	1.62	33.36 (	.50	64.60	2.42 4	3.63 0.5	51 81	.40 2.	80 34.7	0.54
	Total n	iconhytoi	nlankton a	hindan	e								Piconhy	vtonlan	kton bic	sseuu							
	4	on fundament											ndoor v	miden									
	Contro	-			Mussel				Cage				Contro	_			Mussel			Ca	ge		
	Surface		Bottom		Surface		Bottom		Surface		Bottom		Surface		Bottom	51	Surface	В	ottom	Su	rface	Bot	om
	$n \times 10^7$	$I^{-1} \pm SD$	$^{\mathrm{a}}$ $n \times 10^7  \mathrm{I}^-$	$^{-1}\pm SD$	<sup>a</sup> $n \times 10^7  \mathrm{l^{-1}}$	$\pm SD^{a}$	$n \times 10^7  \mathrm{l^{-1}}$	$\pm SD^{1}$	$n \times 10^7  \mathrm{l}^{-1}$	$\pm SD^{i}$	$n \times 10^7  \mathrm{l}^{-1}$	$1 \pm SD^{a}$	$^{1}\mu gCl^{-1}$	$\pm SD^{a}$	$\mu g C l^{-1}$	$\pm SD^{a}$	lgCl <sup>-1</sup> :	$\pm SD^{a} \mu_{i}$	gC l <sup>−1</sup> ±	SD <sup>a</sup> µg	C1 <sup>−1</sup> ±	SD <sup>a</sup> μgC	$I^{-1} \pm S$
Mar	2.66	1.22	1.34	0.02	2.09	0.31	1.58	0.02	2.72	0.25	1.45	0.08	7.83	3.60	3.93	0.06	6.14 0	, 92 ,	4.64 0.	06 7.	0.0 66	4 4.3	2 0.23
Apr	4.31	0.14	4.71	0.57	4.38	0.82	4.06	0.19	5.18	0.54	4.16	0.06	12.81	0.41	13.87	1.66 1	3.00 2	2.52 1.	2.01 0.	60 15.	31 1.6	1 12.2	3 0.18
May	2.06	0.30	1.18	0.19	1.51	0.15	1.07	0.11	1.11	0.25	1.47	0.35	6.12	0.89	3.57	0.57	4.48 (	0.47	3.15 0.	32	36 0.6	6.4 6.7	2 1.0
unr	<del>1</del> .24	0.05	0.80	0.03	1.08	0.08	0.00	17.0	07.7	0.00	787	60.0	4.34	0.50 0000	7.07	0.10	5.24	77.0	2.98 0.	9.5 0. 20 1.0	0/ I.S		5 0.4
	4.30 00 c	87.0	4.10	0.38	4.9/	0.41	5.5/	0.57	4.33	18.0	4.31	0.32	11.70	0.85	14.21	1 36 1	1 00.0	1 /S. 1	.I 0C.U	58 15. 00 14	18 2.0	13.0	7 0.8
Sup of the	20.C	67.0	4.02	00.0	4.20	0C.U	1.01	0.49	4./4	00.0	07.0	01.0	0.00	1.10	14.54	- 0C0	1 10.2	1 0/.1	+.07 U.	با 14.	141	0.01	0.0 2
P. O. Nov	26.6	0.0	5.10 0.71	0.08	44-7 1 18	0.04	16.2	60.1 0.07	2.34 1 48	00.0 80 0	5.08 0.83	0.10	9.89 3.03	0.41	9.10 2.43	0.28 0.50	3.61 0	10.0	. 1 CC.S	 	-0 00 	5 10.5 5 2 C	0.4 0.0
CI CDec	0.73	0.06	0.92	0.12	1.00	0.14	0.92	0.01	1.01	0.08	1.00	0.05	2.29	0.29	3.00	0.50	3.49 0		2.90 0.	12 3.	34 0.5	0 3.5	8 0.3:
Jun 1	10.1	0.09	0.94	0.08	1.09	0.04	1.02	0.05	0.98	0.06	0.93	0.11	3.11	0.40	2.82	0.26	3.95 0	.20	3.36 0.	41 3.	13 0.3	0 3.1	1 0.54
Feb	Qx ₹0.80	0.06	0.33	0.04	0.75	0.09	0.48	0.02	0.87	0.07	0.29	0.03	2.53	0.02	0.98	0.13	2.36 0	.38	1.77 0.	19 2.	60 0.2	4 1.0	2 0.2
avg	P,SED2.33	H.O	2.09	0.05	2.24	0.08	1.94	0.09	2.46	0.08	2.22	0.03	7.04	0.31	6.29	0.16	6.82 6	0.23	5.15 0.	15 7.	72 0.2	8 6.8	7 0.09

 $^{b}SE = Standard Error.$ 

SD = Standard Deviation.

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DIN concentrations were significantly higher only in the mussel area, while DIP in the fish farm area was, on annual average six times higher than in the control. These values are in good agreement with higher  $PO_4$  values reported by Pitta et al. [16] in waters around cage farms in Greek coastal waters. Moreover, Wu et al. [25] observed a decrease in dissolved oxygen and an increase in ammonia, inorganic phosphate, nitrate and nitrite only at sites with poor tidal flushing and high stocking density.

The present study confirms previous findings as no significant differences between farm and control sites were observed in terms of chlorophyll-*a* concentrations (here utilised as a measure of phytoplankton biomass). However, it is possible that these conclusions result from a wrong background hypothesis, based on the simple expectation that primary production will be enhanced by increased release of nutrients. Moreover, water dynamics in the study area (current velocities ranged from 8 to  $10 \text{ cm s}^{-1}$ , during the study period) contributes to dissipate or disperse the inputs originated from the mussel and fish farms.

Comparing surface DOC concentrations at the Control and Cage site we observed significantly higher values around the fish farm. This result is not surprising since the release of organic solutes associated to both feed pellet and fish excretion is expected. The fact that the Mussel site does not display any increase of DOC concentrations, further confirm the specificity of this impact for fish cage areas. In contrast, BPC concentrations were similar to those reported in most coastal sites of the Mediterranean Sea [26]. In this regard, it is interesting to remark that increased organic loads in the water column were dealt only with the dissolved phase, whilst no significant differences were observed comparing the quantity of suspended particulate material (i.e., particles of diam. comprised from 0.4 to 200 µm) between Cage station (or Mussel station) and the Control. Similar lack of POC response to farm biodeposition has been reported by Pitta et al. [16]. All these indications suggest that POM (in the present study expressed as BPC) or POC values fail in establishing relationships with biodeposition and are not good indicators of organic enrichment along the water column due to farming activities.

This finding might also have important implication in the definition of the trophic conditions. Defining the trophic state in the marine environment is always a difficult task as it is not clear whether we refer to nutrient concentrations, autotrophic biomass or particulate fluxes [27]. The results of this study clearly point out that changes in the trophic state can occur simply as a result of changes in DOC content, a variable so far never taken into account for this kind of study.

Total picoplankton counts  $(10^8 - 10^9 \text{ cells } l^{-1})$  were similar to those obtained in other coastal areas [28]. However, we observed an increase in the picoplankton density at the Cage station, related to the increased DOC concentrations. Also, picophytoplankton abundance and biomass were in agreement with values reported for coastal, estuarine and brackish environments [29], but did not display significant differences among stations, thus indicating that only the heterotrophic component was affected by DOC input. From the analysis of the different microbial variables it is possible to conclude that none of the microbial autotrophic components considered in this study (picophytoplankton, including autotrophic eukaryotes and cyanobacteria and large-size autotrophic cells such as microphytoplankton) are good descriptors of farm disturbance in this environment.

# 5. Conclusions

The comparative analysis of cage and mussel farms revealed that mussel cultures induced a considerably lower disturbance. Evident changes were observed only in terms of increased inorganic nitrogen concentrations along the water column, compared to both control and fish farms. The organic input from the mussel farm is known to produce enhanced organic matter concentrations on sediments [2]. Faeces and pseudofaeces are characterised by a large bioavailability to microbial assemblages and by rapid degradation rates [13,30]. All these factors certainly contribute to a rapid turnover of the organic biodeposits under the mussel farms and to the massive release of nutrients at the water-sediment interface, which, in turn, can modify the nutrient budgets around the farms [5]. Mussel biodeposits could be responsible for the enhanced picoplankton density and biomass observed at the mussel site. However, again, such density increase was limited to the heterotrophic components, as chlorophyll-a concentrations and picophytoplankton values did not change compared to the control.

By contrast water enrichment due to organic and inorganic components loading from fish farms was evident only in terms of DOC concentrations. Such change determined an increase of the heterotrophic fraction of picoplankton, while picophytoplankton, did not display differences among fish (or mussel farms) and Control site.

The result and conclusions can be resumed in three major points as follow:

• Temporal changes observed for most variables, including those more evidently effected by fish and mussel cultures, followed typical seasonal patterns, characterising also the Control site.

- The farming impact over the water column must be searched more in terms of a continuous input of DOC and nutrients increasing the background levels, rather than as a process able to modify the natural seasonal variability.
- The comparative analysis of cage and mussel farms impact on water column, revealed that mussel cultures induced lower disturbance in open environment, as that investigated.

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