The effect of mariculture facilities on biochemical features of suspended organic matter (southern Tyrrhenian, Mediterranean)

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Abstract

A comparison of a Mediterranean aquaculture impacted area and control areas was made to assess the effect of fish farm waste discharge on the biochemical features of the water column. Trophic variables commonly used in marine ecology such as total suspended matter, suspended chlorophyll-*a*, biochemical features of particulate organic matter (proteins, lipids and carbohydrates) and biopolymeric carbon were chosen as the best descriptors of trophic conditions. An initial analysis of data from the impact area was carried out in order to test the effect of farm waste using a gradient of distances downstream from the fish farm cages (50 m, 300 m, 1000 m). The results were then compared with a control site 750 m upstream. Subsequently, the cage data set was asymmetrically compared with data from five controls collected some years before, when no aquaculture activity was present in the Gulf. The analysis revealed differences in chlorophyll-*a*, carbohydrates and some trophic ratios between the farm impact area and the controls taken upstream. A clear pattern of trophic enrichment of the water column around the fish farm was evidenced since concentrations in the sites increased along with their distance from the cages. The downstream sites overall were significantly different, trophically speaking, from the five control areas, while the trophic variables of the upstream control were not different from the external controls. Results showed that fish farm facilities provided an organic enrichment of the water column up to at least 1000 m downstream from the cages, producing a deviation of trophodynamics from normal ambient conditions.
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1. Introduction

Large aggregations of aquatic organisms, such as cultivated fish and shrimp, produce large quantities of allochthonous dissolved and particulate organic matter (Wu, 1995; Beveridge, 1996) capable of causing environmental problems (Islam and Tanaka, 2004). The quantity of organic waste produced is a function of aggregated biomass, feed conversion and type of cultivated organism; the quality of allochthonous fish farm organic matter is a function of feed composition and faecal waste generation. Such organic matter, once in the surrounding environment, represents a further allochthonous source of nutrients differently available to pelagic and benthic systems. In the water column, dissolved and particulate organic waste undergoes the effects of hydrodynamic forces able to remove particulate waste from the emission point, amplifying settling times in underlying sediments (Sarà et al., 2004). Sediments receiving this "organic rain" constantly produced over time, become the biological and ecological memory of potential perturbations. The potential effects of organic surplus

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waste coming from fish farm facilities on sediments have been analysed in many papers highlighting, in most cases, the detrimental effects on bacteria (La Rosa et al., 2001), meiofauna (Mirto et al., 2002), macrofauna (Findlay et al., 1995; Karakassis et al., 2000) seagrass dynamics (Cancemi et al., 2003), sediment chemistry (Carroll et al., 2003; Sarà et al., 2004) and benthic food web variability (Ye et al., 1991; Vizzini and Mazzola, 2004). In contrast, information in the current literature on water column tropho-chemistry is rather scant. Only a few papers have considered trophic variables in the water column as potential descriptors of the detrimental effects of waste loading (Pitta et al., 1999; La Rosa et al., 2002; Alongi et al., 2003; Burford et al., 2003; Sarà et al., 2004). For example, shrimp and fish cultivation in Malaysian estuaries and in Australia produced some detrimental effects on the water column, showing a clear pattern of diminishing values of all particulate variables between farm and reference sites (McKinnon et al., 2002a,b; Alongi et al., 2003; Burford et al., 2003). Similarly, in the Mediterranean, suspended bacteria, phytoplankton, and particulate organic matter were significantly altered by organic discharge in proximity of the emission points, but did not consistently vary between cage and non-cage waters (Pitta et al., 1999; La Rosa et al., 2002). Thus, most studies revealed only a negligible effect on the water column between farm and reference sites and never more than 100 or 200 m from the centre of farm emissions. Even though the water column seems to be an aleatory system subject to a high stochastic variability and highly site-specific (Cromey et al., 2002a,b; Sarà et al., 2004), its dynamics should not be neglected in monitoring studies. Indeed, hydrodynamic forces play an important role in displacement, distribution, canalisation and transfer of organic matter

through the biota (Alongi, 1998) far from production sites (Silvert and Cromey, 2001; Cromey et al., 2002a,b; Sarà et al., 2004). The study of sediment chemistry processes, and of the dynamics and structure of benthos caused by organic waste on sediments, should not disregard consideration of the dynamics and effects of organic wastes on the water column. In a similar vein, the aim of the present paper is: (1) to test whether organic waste loading affected water column organic matter as a function of the downstream distance from the centre of a Mediterranean fish farm; and (2) to test if particulate organic matter quality and quantity in the putative impact area were different, over time, in respect to ambient concentrations.

2. Materials and methods

The study was carried out between May and July 2001 off the northern coast of Sicily, in the Gulf of Castellammare (Latitude 38° 02′ 31″ N; Longitude 12° 55′ 28″ E; Fig. 1). The area is seasonally influenced by terrigenous-continental inputs (Sarà et al., 1998, 2004), which originate from nearby streams. In late spring 2000, four submersible cages (Floatex, Italy; volume = 1000 m^3) were positioned in the western part of the Gulf (Latitude 38° 02′ 60″ N; Longitude 12° 55′ 60″ E) and moored on the bottom at a depth of about 30 m, 1 nm off the coast. The hydrodynamic regime of the cage area (Sarà et al., 2004) is characterised by a dominant current coming from the third and fourth quadrants (along a west–east axis) for most of the year. The cages were filled in July 2000 with 54,000 specimens of *Dicentrarchus labrax* (initial total length of 160.9 ± 17.0 mm and total weight of 50.6 ± 16.3 g) and 69,500 specimens of *Sparus aurata* (initial
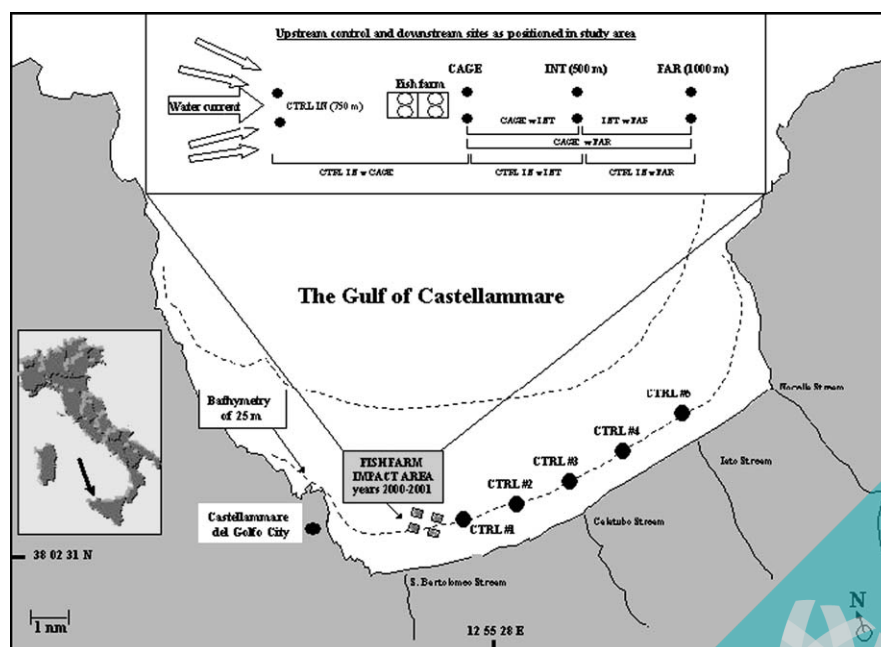

Fig. 1. The map of the study area, the Gulf of Castellammare (Sicily, Mediterranean), showing the area of the fish cages (fish farm impact area – years 2000–2001) and five control areas from which data were collected during oceanographic cruises carried out five years before.

total length of 129.9 ± 15.9 mm and total weight of 33.6 ± 12.9 g). The total initial transplanted biomass was 5047 kg over four cages. Farming was carried out until summer 2001, when the total biomass reached was about 33,800 kg. Throughout the farming period, the average food conversion ratio was estimated to be about 2.5. The total supplied feed (different types of commercial feed produced by BioMar, France and Hendrix, Italy) was about 80 tons.

2.1. Sampling and biochemical analysis

To address the question of whether organic discharge coming from aquaculture facilities had effects on the biochemical characteristics of particulate organic matter (POM), sites were positioned in order to obtain samples with the highest degree of organic waste deposition coming from cages. Six sampling sites, at different distances from the cages, were located downstream along the main axis of the water current (Fig. 1): two sites were within a radius of 50 m downstream (hereafter referred to as CAGE sites); two sites were situated 500 m downstream (hereafter referred to as INT sites); and the final two sites were about 1000 m downstream from the cage centre (hereafter referred to as FAR sites). An internal control site (hereafter referred to as CTRL IN) was positioned upstream from the farm (at about 750 m). In addition, to allow comparison with normal ambient concentrations in the Gulf, data were used from reference locations (hereafter referred to as CTRL OUT sites) collected during oceanographic cruises (Manganaro et al., 1998) carried out some years before the study cages were moored in the Gulf (2000). At that time no aquaculture activity was present in the Gulf, so it was assumed that the cruise data could be used to represent the natural trophic variability of the study area because of the absence of anthropogenic disturbance or organic enrichment coming from aquaculture facilities. CTRL OUT locations were randomly chosen throughout the whole Gulf of Castellammare at the same bathymetry of the impact area (Fig. 1).

In each location, water samples, collected using a 10-l Niskin bottle, were brought back to the laboratory and processed within a few hours. Water samples were screened through a 200 μm mesh net in order to remove large zooplankton and debris. Sub-samples (500–2000 ml) were filtered onto pre-washed, precombusted ($450°$, 4 h) and pre-weighed Whatman GF/F filters (0.45 μm nominal pore size). For each replicate, the total suspended material (TSM, mg l^{-1}) determination was carried out gravimetrically after desiccation (105 °C, 24 h) using a Sartorius M200 balance (accuracy $\pm$ 1 μg), while chlorophyll-a (CHLa, μg l^{-1}) was determined according to Lorenzen and Jeffrey (1980). Particulate carbohydrate concentrations (CHO, μg l^{-1}) were measured according to Dubois et al. (1956) and reported as glucose equivalents. Particulate proteins (PRT, μg l^{-1}) were determined according to Hartree (1972) and reported as bovine serum albumin (BSA) equivalents. Particulate lipid concentrations (LIP, μg l^{-1}), measured according to Bligh and Dyer (1959) and Marsh and Weinstein (1959), were reported as tripalmitine equivalents. The sum of carbohydrates, proteins and lipids is

indicated as particulate organic matter (POM, μg l^{-1}) and were converted to carbon equivalents using the coefficients 0.40, 0.49 and 0.75, respectively (Pusceddu et al., 2003). The sum of the carbon equivalents of the three main components of POM is indicated as total biopolymeric particulate organic carbon (BPC, μg C l^{-1}; Pusceddu et al., 2003). The CHLa/BPC (the concentration of chlorophyll-a converted into carbon units using 52 as the conversion factor – Nival et al., 1972), PRT/CHO and POM/TSM ratios (Widdows et al., 1979; Navarro et al., 1993; Sarà et al., 1998), were used as tools for gathering information about the qualitative features of the particulate matter.

2.2. Experimental rationale and statistical analysis

The rationale for the design presented in this study was to test, firstly, the variability within the fish farm impact area, and then to compare asymmetrically the whole impact area with the five control areas, assuming that any impact would cause a greater difference in variability to emerge between impact and control areas than the differences in natural variability occurring among the control areas.

2.3. Partial ANOVAs to test differences among distances in IMPACT area

This study was designed to address the question of whether any impact will cause more differences between experimental and control locations than the differences that occur naturally among control locations separated by the same distance (Kelaher et al., 1998). To do so six partial three-way ANOVAs were used (CTRL IN vs CAGE; CTRL IN vs INT; CTRL IN vs FAR; CAGE vs INT; CAGE vs FAR; INT vs FAR) to verify the hypothesis that particulate organic matter (POM) varied as a function of the distance gradient from the centre of cages (viz. the point-disturbance source) and with respect to an upstream control (CTRL IN). In each of the six ANOVAs, distance was treated as fixed and orthogonal (two levels; DIST), while sites (two for each distance) were treated as random (two levels; SITE) and nested in DIST. Two replicates were randomly collected at each site. PERIOD (two levels; PER → spring and summer) was treated as fixed and orthogonal.

2.4. Asymmetrical ANOVAs to test the difference between and within IMPACT and external reference control locations

Once the variability within the impact area was tested to study variance among its locations (downstream distances vs upstream control) in the two main periods (spring and summer), an asymmetrical ANOVA was carried out to test differences among the locations in the impact area (CTRL IN, CAGE, INT and FAR) and in the five reference control areas (CTRL OUT). Thus a series of three-way asymmetrical ANOVAs were run in order to test the null hypothesis of whether trophic variables at each distance in the impact area

were equal to those of the five control areas. In this analysis, locations (LOC; CTRL IN or CAGE or INT or FAR vs CONTROL OUT; one and five levels, respectively) were treated as a fixed factor, sites in each area (SITE nested in LOC; two levels) as a random factor, and PERIOD (PER; spring and summer, two levels) as a fixed factor. Two sampling replicates were taken randomly at each site. For each analysis the heterogeneity of variances was tested using Cochran's C test prior to the analysis of variance. The Student−Newman−Keuls (SNK) test allowed the appropriate mean comparison.

3. Results

3.1. Differences among distances in the IMPACT area

The statistics of trophic variables measured in the impact area are summarised in Table 1, while Table 2 reports partial ANOVA results comparing impact locations throughout the study periods. Though concentration of total suspended matter was on average $7.9 \pm 6.7 \, \mathrm{mg \, l^{-1}}$ within the impact area, it was not different at the locations. A higher accumulation $(12.1 \pm 13.2 \, \mathrm{mg \, l^{-1}})$, however, was detected at 300 m from the edge of the cages (INT sites; see Table 1). Particulate

Table 1

Statistics of trophic variables within impact sites ($\pm$se = standard error for means; TSM = total suspended matter, $\mathrm{mg \, l^{-1}}$; C-CHLa = chlorophyll-a carbon, $\mu\mathrm{g \, l^{-1}}$; C-LIP = carbon from lipids; $\mu\mathrm{g \, l^{-1}}$; C-PRT = carbon from proteins; $\mu\mathrm{g \, l^{-1}}$; C-CHO = carbon from carbohydrates; $\mu\mathrm{g \, l^{-1}}$; BPC = biopolymeric carbon as a sum of C-LIP, C-PRT and C-CHO; $\mu\mathrm{g \, l^{-1}}$; C-CHLa/BPC = chlorophyll-a carbon by biopolymeric carbon ratio; C-PRT/C-CHO = carbon proteins by carbohydrate ratio; POM/TSM = particulate organic matter [as a sum of lipids, proteins and carbohydrates expressed as $\mathrm{mg \, l^{-1}}$] by TSM ratio; PRT/CHO = proteins by carbohydrates ratio)

	CTRL IN				IMP-CAGE			
	Spring		Summer		Spring		Summer	
	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se
TSM	5.1	0.5	1.8	0.8	10.7	13.9	2.8	1.1
C-CHLa	0.5	0.0	0.5	0.0	5.5	1.6	17.5	20.8
C-LIP	67.0	30.3	82.6	45.5	50.9	37.1	50.0	31.3
C-PRT	32.6	6.4	44.0	10.6	56.7	14.1	55.0	21.1
C-CHO	4.0	1.4	7.1	1.3	18.7	3.0	18.4	6.1
BPC	103.6	36.2	133.7	54.3	126.3	50.9	123.5	24.9
C-CHLa/BPC	1.6	0.4	1.4	0.4	4.6	1.5	13.1	13.3
C-PRT/C-CHO	8.9	3.5	6.4	2.3	3.0	0.6	3.9	3.5
POM/TSM	3.3	1.4	15.4	11.3	6.5	6.8	8.6	2.5
	IMP-INT				IMP-FAR			
	Spring		Summer		Spring		Summer	
	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se
TSM	21.4	33.1	2.7	0.8	3.6	1.3	11.1	16.7
C-CHLa	7.3	2.5	4.0	2.6	31.8	21.0	26.0	30.4
C-LIP	55.4	25.3	21.1	8.3	37.4	26.1	53.8	27.8
C-PRT	48.2	29.7	45.3	6.0	56.6	9.1	41.0	11.6
C-CHO	23.0	5.4	25.1	4.9	28.8	11.0	29.2	24.5
BPC	126.6	50.8	91.6	14.5	122.8	39.0	124.0	52.7
C-CHLa/BPC	6.2	1.7	4.5	3.4	30.2	23.9	25.7	28.9
C-PRT/C-CHO	2.1	1.1	1.9	0.4	2.1	0.4	1.9	0.9
POM/TSM	3.7	3.4	7.0	1.4	7.1	2.5	7.2	4.7

Table 2

Outcome of partial ANOVAs carried out within impact area comparing internal control (CTRL IN), cage (CAGE), intermediate (INT) and far (FAR) locations during spring vs summer periods. Only variables which significantly varied are reported (statistical parameters of ANOVA − mean squared, Fisher value and P-level − are not reported for synthesis and brevity)

Location	CTRL IN	CAGE	INT	FAR
CTRL IN vs	−			
CAGE vs	C-CHLa; C-CHO	−		
INT vs	C-CHO; C-PRT/C-CHO	C-PRT; C-CHO; C-PRT/C-CHO	−	
FAR vs	C-CHL; C-CHO; C-PRT/C-CHO; C-CHL/BPC	C-PRT; C-PRT/C-CHO	C-CHL; C-CHO; C-CHL/BPC	−

organic matter (POM) was on average $214.7 \pm 67.0 \, \mu\mathrm{g \, l^{-1}}$ (in terms of biopolymeric carbon about $119.0 \pm 39.0 \, \mu\mathrm{g \, C \, l^{-1}}$), ranging between 111.0 (about $57.0 \, \mu\mathrm{g \, C \, l^{-1}}$) and $376.0 \, \mu\mathrm{g \, l^{-1}}$ (about $201.0 \, \mu\mathrm{g \, C \, l^{-1}}$). It represented, on average, only $7.4 \pm 5.9\%$ of the total suspended matter (TSM) and did not show significant differences among the sites at the various distances. Biopolymeric carbon, POM converted to carbon units, and its fractions did not show any difference when comparing locations and periods. Chlorophyll-a concentrations were lower than $1 \, \mu\mathrm{g \, l^{-1}}$ (i.e. $52 \, \mu\mathrm{g \, C \, l^{-1}}$) throughout the study period and at all locations. Significant differences in chlorophyll-a (partial ANOVA, $p < 0.05$; Table 2), however, were detected between CTRL IN vs CAGE, CTRL IN vs FAR and INT vs FAR (Fig. 2). Organic carbon (C-CHLa) coming from phytoplankton represented about $8.2 \pm 11.3\%$ of the biopolymeric carbon in the study area (see C-CHLa/ BPC ratio values; Table 1), and it was different between CTRL IN and FAR and between INT and FAR (Table 2). Carbohydrates varied between 2.6 and $66.0 \, \mu\mathrm{g \, C \, l^{-1}}$ (on average $17.9 \pm 9.4 \, \mu\mathrm{g \, C \, l^{-1}}$), showing a clear distance gradient from the cage to the far sites (Table 2). In particular, CAGE carbohydrate concentrations were different between CTRL IN and all distance locations, as well as between each distance (partial
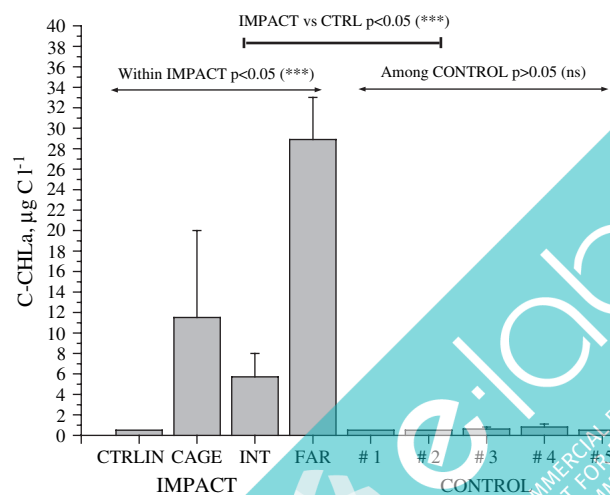

Fig. 2. Suspended chlorophyll-a expressed as carbon equivalents (C-CHLa) comparing impact area and five controls (impact = impact area; CTRL = control areas from 1 to 5) (ANOVA SNK outcome is reported).

ANOVAs, $p < 0.05$; Fig. 3). Proteins showed a peak at CAGE, which was significantly different with respect to INT and FAR, while lipids, did not show any significant differences. The C-PRT/C-CHO ratio ranged between 2.0 ± 0.1 and 7.6 ± 1.8, decreasing significantly from CTRL IN to INT and FAR sites (partial ANOVA, $p < 0.05$; Table 2; Fig. 4).

3.2. Difference between the IMPACT area and external reference control locations

The statistics of trophic variables measured in the five controls and in the impact area and the outcome of an asymmetrical ANOVA done to test the null hypothesis of equality among them are reported in Tables 3 and 4. The overall pattern was that, apart from TSM, proteins and BPC, almost all trophic variables showed differences between CTRL OUT locations and the distance gradient locations from the cages, while in no case were differences observed between CTRL IN and CTRL OUT and no differences were detected among CTRL OUT locations. The impact area was slightly different, from a quantitative point of view, from the control areas as total suspended matter concentrations were twice as high as in the controls (8.7 ± 7.3 mg l^{-1} and 3.7 ± 2.1 mg l^{-1}, respectively), although they did not significantly differ. Carbon chlorophyll-*a* concentration was about 25 times higher in the farm area than in the control locations. Chlorophyll-*a* showed a strong significant difference (Fig. 2) between CTRL OUT and CAGE to FAR sites, while no differences were detected within CTRL IN. On the contrary, lipid concentration was significantly lower in the impact sites than in the control sites, but was not different within CTRL IN (figure not reported). Carbohydrates (Fig. 3) estimated in the farm area were different from reference control locations, being about 2.5 times higher within the impact area than outside it. Also chlorophyll-*a* by BPC ratio showed significant differences when comparing the impact area with control out locations (figure not reported), being almost 13 times higher in the impact sites. A different
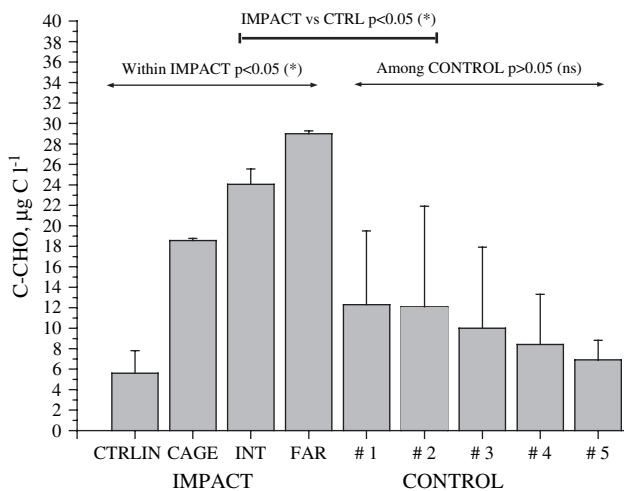

Fig. 3. Total particulate carbohydrates expressed as carbon equivalents (C-CHO) comparing impact area and five controls (impact = impact area; CTRL = control areas from 1 to 5) (ANOVA SNK outcome is reported).
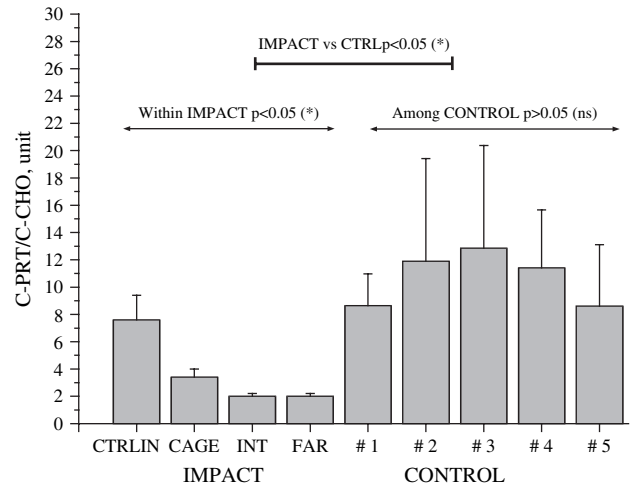

Fig. 4. C-PRT by C-CHO ratio comparing impact area and five controls (impact = impact area; CTRL = control areas from 1 to 5) (ANOVA SNK outcome is reported).

picture was observed for protein by carbohydrate ratio whose values were higher in control out locations (about 3.5 times higher) than in the impact sites (Fig. 4).

4. Discussion

The present study tested whether organic enrichment produced by fish farm facilities had any effect on organic matter features in the water column. The results showed that a fish farm in which about 40 tons of fish biomass were cultured over time, led to a biochemically detectable organic enrichment of the water column. The first part of the study considered variability among within impact locations as a function of different distances from the cages. From this, a general trophic enrichment of the water column was evident because most of the variables varied between the internal upstream control (CTRL IN) and the locations downstream from the cages. Almost all the trophic variables significantly varied among the downstream locations according to a clear pattern in which the concentrations increased in the sites farthest from the cages. Chlorophyll-*a* also showed a significant pattern, increasing in concentration in locations farthest from the emission point. Nutrient loading and organic discharge seem, in the study of the Gulf of Castellammare, to have an effect on the dynamics of the water column up to 1 km from the cages. The asymmetrical analysis also demonstrated that the general trophic conditions generated by fish farms in the Gulf of Castellammare deviated from the usual southern Tyrrhenian ambient conditions. Accordingly, concentrations of chlorophyll-*a* (on average about $0.2-0.4$ μg l^{-1}) were significantly higher (25 times) than normal ambient conditions measured before the mooring of the cages. A similar result was also obtained in a Greek fish farm where chlorophyll-*a* concentration was about 20 times higher at the cage than at the reference site (Pitta et al., 1999). Nevertheless, concentrations measured in the study area, were still lower than the mean Mediterranean value (about $0.5-1$ μg l^{-1}; Margalef, 1985;

Table 3

Statistics of trophic variables in impact (considering only downstream locations, CAGE, INT and FAR) and external control areas ($\pm$se = standard error for means; TSM = total suspended matter, mg l^{-1}; C-CHLa = chlorophyll-a carbon, μg l^{-1}; C-LIP = carbon from lipids; μg l^{-1}; C-PRT = carbon from proteins; μg l^{-1}; C-CHO = carbon from carbohydrates; μg l^{-1}; BPC = biopolymeric carbon as a sum of C-LIP, C-PRT and C-CHO; μg l^{-1}; C-CHLa/BPC = chlorophyll-a carbon by biopolymeric carbon ratio; POM/TSM = particulate organic matter [as a sum of lipids, proteins and carbohydrates expressed as mg l^{-1}] by TSM ratio)

| | IMPACT | | CTRL #1 | | CTRL #2 | | CTRL #3 | | CTRL #4 | | CTRL #5 | |
	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se	mean	$\pm$se
TSM	8.7	7.3	3.8	2.2	3.6	2.4	3.6	2.2	3.8	2.0	3.7	2.1
C-CHLa	15.4	11.7	0.5	0.0	0.5	0.0	0.6	0.2	0.8	0.3	0.5	0.0
C-LIP	44.8	13.2	54.0	12.8	114.4	103.8	98.8	89.2	67.5	30.3	62.9	39.3
C-PRT	50.5	6.6	100.8	55.1	89.8	26.3	94.1	38.9	84.7	27.9	56.7	34.3
C-CHO	23.9	4.7	12.3	7.2	12.1	9.8	10.0	7.9	8.4	4.9	6.9	1.9
BPC	119.1	13.6	167.1	71.1	216.3	117.1	203.0	95.2	160.7	45.2	126.4	54.2
C-CHL/BPC	14.1	11.3	1.2	0.3	1.1	0.2	1.2	0.3	1.4	0.2	1.5	0.5
C-PRT/C-CHO	2.5	1.6	8.6	2.3	11.9	7.5	12.9	7.5	11.4	4.2	8.7	4.5
POM/TSM	6.7	1.6	12.5	13.2	18.4	18.0	17.3	18.0	9.3	4.6	8.4	7.1

Pitta et al., 1999; Sarà et al., 2004) and only negligible in respect to the threshold value needed to avoid eutrophication recommended for northern European waters (10 μg CHLa l^{-1}; CSTT, 1994). The organic enrichment produced from fish farm waste in the study area appears to be still below the minimal threshold necessary to cause undesirable biological consequences, apart from a likely increase in nutrient levels able to enhance the phytoplankton biomass. Similar results were consistent with another study carried out in the same area reporting on the isotopic composition of POM in fish farm waters (Sarà et al., 2004). The POM isotopic signature was altered and constrained by organic nitrogen from fish farm waste up to the 300 m sites and probably beyond. Nevertheless, from the present study, particulate protein concentrations could explain that the result was not significantly altered in the impact locations with respect to the control ambient areas. A

Table 4

Outcome of asymmetrical ANOVA carried out for testing the null hypothesis of equality between (a) CONTROL OUT sites each distance in within-impact and (b) the effect of periods in particulate organic matter patterns. For synthesis, we report only the main fixed effects and not the random effect and only Fisher values and correspondent significance levels. Rationale of experimental design and acronym explanations are reported in Section 2 (significance levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = non-significant difference $-$ $p > 0.05$)

| (a) | | TSM | | C-CHLa | | C-LIP | | C-PRT | |
		F	P	F	P	F	P	F	P
CTRL OUT vs	CTRL IN	0.01	ns	5.04	ns	0.02	ns	2.25	ns
	CAGE	1.38	ns	16.55	**	7.12	*	0.89	ns
	INT	3.31	ns	46.94	***	7.58	*	1.52	ns
	FAR	1.38	ns	470.21	***	7.26	*	1.35	ns
AMONG	CTRL OUT	0.00	ns	6.73	ns	1.72	ns	0.30	ns
		C-CHO		BPC		C-CHL/BPC		C-PRT/C-CHO	
		F	P	F	P	F	P	F	P
CTRL OUT vs	CTRL IN	2.35	ns	2.72	ns	1.80	ns	3.11	ns
	CAGE	4.44	ns	2.24	ns	17.08	*	19.09	***
	INT	8.04	*	3.68	ns	56.76	***	27.89	***
	FAR	9.18	*	2.38	ns	474.60	***	27.85	***
AMONG	CTRL OUT	0.21	ns	1.16	ns	1.45	ns	1.41	ns
(b)		TSM		C-CHLa		C-LIP		C-PRT	
		F	P	F	P	F	P	F	P
PERIOD $\times$ CTRL OUT vs	CTRL IN	0.01	ns	0.21	ns	0.03	ns	0.01	ns
	CAGE	3.02	ns	4.77	ns	1.00	ns	0.04	ns
	INT	3.08	ns	4.18	ns	0.35	ns	0.01	ns
	FAR	3.02	ns	1.33	ns	4.10	ns	0.04	ns
		C-CHO		BPC		C-CHL/BPC		C-PRT/C-CHO	
		F	P	F	P	F	P	F	P
PERIOD $\times$ CTRL OUT vs	CTRL IN	0.24	ns	0.00	ns	0.04	ns	0.34	ns
	CAGE	0.00	ns	0.05	ns	4.76	ns	0.03	ns
	INT	0.00	ns	0.11	ns	3.83	ns	0.36	ns
	FAR	1.13	ns	1.32	ns	0.94	ns	0.68	ns

possible explanation of this result is that if an alteration of the nitrogen pool occurred, surplus nitrogen due to waste discharge could easily be metabolised by bacteria and phytoplankton (La Rosa et al., 2002). Carbohydrates, composing fish feed, are usually more refractory for biological consumption than proteins (Mann, 1988), so that their consumption in the water column would be slower than proteins, causing accumulation in the water column, and consequently in the underlying sediments (Sarà et al., 2004) of locations far from the cages. Apart from carbohydrates, relationships between carbon chlorophyll-*a* and total biopolymeric carbon changed in the impact area. In ambient conditions (see control values; Table 3), carbon chlorophyll-*a* represented not more than 2% of the total BPC over time (Sarà et al., 1998), while in cage waters it increased by about 12–14 times. Such a result further validates the aforementioned hypothesis that a constant input of organic waste from the cages did alter fresh biomass concentrations, but not other characteristics of suspended organic bulk.

Present results can be justified mainly by a "hydrodynamic factor". In the Gulf of Castellammare, the mean water current velocity throughout the year is about 10–12 cm s^{-1}. Such conditions were able to generate a constant downstream flux of particulate material. According to recent models of waste dispersal (Cromey et al., 2002a,b) and behaviour in the water of fish pelleted feed (hardness, friability, settling velocity, relationship with salinity and temperature; Chen et al., 1999), the hypothesis of an effect of organic enrichment of the water column even at several hundred meters from the cages can be corroborated. In addition to this, some recent insights about the role of escaped and wild fish living around fish farms can be invoked to further justify an amplification of organic matter exportation processes towards sites far from the cages (Dempster et al., 2002; Sarà et al., 2004; Vita et al., 2004). Vita et al. (2004), in particular, showed that about 80% of the particulate organic matter produced by cultivated fish may be consumed before it settles on sediment far from the cages, and that significant changes in the nutrient quality of the organic matter exported could also be due to consumption by wild fish.

Results obtained by the present study are in contrast with the current literature, although only a few studies have considered the biochemical features of POM among descriptors of environmental fish farm effects. The monitoring of traditional water quality variables has verified that the downstream impact of fish and shrimp effluent is only measurable in close proximity to the waste emission point (Hensey, 1991; Samocha and Lawrence, 1995; Pitta et al., 1999; Tovar et al., 2000a,b; Trott and Alongi, 2000, 2001; Jones et al., 2001; La Rosa et al., 2002; McKinnon et al., 2002a,b). Fish farm organic loading in the southern Tyrrhenian affected an area larger than that usually influenced by fish farms worldwide. Since the first reviews on this topic (see Beveridge, 1996, as an example), it was concluded that in marine waters the response of trophic components to hypernutrification was evident only in the micro-tidal and low-salinity marine environment and in sheltered bays. Also, since the earliest papers (Gowen and

Bradbury, 1987), sampling protocols have considered only one or two reference sites not farther than 100 or 200 m from the organic loading emission points. To our knowledge, it seems that there is no well defined evidence in the current literature of a clear waste product effect over 100–300 m from the focus of cage emissions (see Pearson and Black, 2000 for a review). The present results clearly contradict this general view, making it necessary to reconsider the choice of descriptors of processes of the water column that, when combined with robust sampling programs, would enable comparisons between putatively impacted and control locations over different spatial scales.

Even though some effects of organic loading on the water column have been detected up to 1000 m from the cages and some differences in ambient conditions, no "impact" which implies both biological consequences and environmental costs change was detected. This agrees with Pitta et al. (1999) when they discuss the concept of impact and raise the need to review it. In the present system, an alteration of normal organic enrichment of the water was only detected but no evident undesirable effects of that enrichment. Thus, highly mixed (water current velocity > 10 cm s^{-1}) oligotrophic waters of the study area seem to be able to accommodate organic waste (over than 40–50 tons; Sarà et al., 2004) coming from fish farm facilities, converting it into fresh biomass, still maintaining the system under the impact threshold.

Acknowledgements

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