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Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics

G. Sarà[1,*], D. Scilipoti[2], M. Milazzo[1], A. Modica[2]

[1]Dipartimento di Biologia Animale dell'Università, Via Archirafi, 18, Palermo 90123, Italy
[2]Centro Oceanologico del Mediterraneo, Piazzale le del Fante, 40, Palermo 90146, Italy

ABSTRACT: Stable isotopes were used to examine differential effects of fish farm waste on the water column and sediments. To achieve this objective, we chose 3 marine fish farms located along the coast of Sicily (Mediterranean Sea) as point-source disturbances, and a control area. The hypothesis that carbon and nitrogen isotope composition of particulate (POM) and sedimentary (SOM) organic matter varied with increasing distance (from cages to 1000 m) was tested at 3 levels of hydrodynamics: low (mean velocity of current [MVC] ~12 cm s^{-1}), intermediate (MVC ~22 cm s^{-1}), and high (MVC ~40 cm s^{-1}). Different isotopic signals from allochthonous (fish waste) over natural (phytoplankton, terrigenous, and sand microflora) inputs allowed identification of the 'spatial effect regime' of fish farming. The increasing water current velocities seem to proportionally enlarge the relative area of influence of the cages, particularly on sediments. At low hydrodynamics, an increasing contribution of terrigenous signals was inferred: POM and SOM showing a depleted gradient of C (ranging from –22.0 to –24.0‰) and N (from 5.0 to 2.0‰). At an intermediate hydrodynamic level, C and N showed a slight increase in waste contribution, particularly in POM ($\delta^{15}N$ from 2.6 to ~4.0‰). At high hydrodynamics, an enriching isotopic gradient ($\delta^{15}N_{POM\text{-}SOM}$ from 1.8 to 4.6‰) suggested a notable contribution of fish waste. Accordingly, the dispersal of waste from the cages seemed to be related to movements at the bottom of the water column, confirming the recently identified role played by resuspension movements.

KEY WORDS: Fish farming · Impact · Water column · Sediment · Stable isotopes · Hydrodynamics · Mediterranean

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INTRODUCTION

In recent years, fish farming activity has increased tremendously along coasts worldwide (Naylor et al. 2000). Environmental impact assessment has been employed to examine the potential disturbance of fish farming on surrounding marine environments (Wu 1995), and several biotic and abiotic descriptors have been used for this. For example, aquaculture-associated effects on the following parameters have been assessed: dynamics of dissolved substances in the water column (Alongi et al. 2003), structure of bacterio-plankton and phytoplankton communities in pelagic (Alongi et al. 2003) and benthic environments (Findlay et al. 1995, Demir et al. 2001), meiofauna (Mirto et al. 2000, 2002) and macrofauna composition (Simenstad & Fresh 1995), and sediment profiles (determined through imagery) (Karakassis et al. 2002).

These studies revealed that impacts of fish farming may be detected from alterations in the structure and composition of natural communities. The extent of the effect of fish farms (namely, Area Zone Effect or AZE) seems to be limited in space (Pearson & Black 2000); however, the role of local hydrodynamics should be taken into consideration (Cromey et al. 2002). In particular, a range of hydrodynamic levels may differentially remove, distribute, and transfer fish farm waste throughout the water column and underlying sediments (Alongi 1996). However, in only a few cases has this been effectively investigated.

*Email: gsara@unipa.it

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Stable isotopes appear to be a promising tool for assessing patterns in dispersal of waste from fish farms (McGhie et al. 2000, Jones et al. 2001, Burford et al. 2003, Sarà et al. 2004, Vizzini & Mazzola 2004). Hobson (1999) argued that stable isotopes are the most powerful tools available to trace organic matter for several fields of ecological research, and others have recommended their use in pollution studies (e.g. Kidd 1998). In this study, the spatial effect of organic enrichment from fish farming was investigated on a regional scale. Carbon and nitrogen stable isotopes were adopted to test: (1) the extension of AZE at different hydrodynamic levels; and (2) differential signatures in the water column (as particulate organic matter [POM] and sedimentary organic matter [SOM]) as a 'memory tracer' of degree of disturbance.

MATERIALS AND METHODS

Study area and sample collection. Our study was conducted from May to June 2001 in 3 areas (Fig. 1) off the coast of Sicily (37°45′N, 13°45′E). The 3 areas are influenced by constant, seasonally fluctuating, terrigenous inputs, which originate from nearby streams.

In each area, a marine fish farm (and with a mean biomass production of ~40 tons of *Dicentrarchus labrax* and *Sparus aurata*) (CEOM 2002) was positioned within a couple of km from the coast and moored on the bottom at a depth of about 26 to 30 m. In all areas, sediments around fish cages were mostly unvegetated. At each location, current meters (RD Instruments) were positioned at intervals of 0.5 m to continuously measure current velocities and directions from surface to bottom at time intervals of 1 h, from autumn to spring. The flow at each site was basically unidirectional, according to a general scheme of water mass circulation in the Tyrrhenian Sea and the Strait of Sicily (Millot 1999, Onken & Sellschopp 2001). Data on the local hydrodynamic regime of the 3 cage locations were characterised by different mean velocities that increased from surface to bottom (Table 1; CEOM 2002). Water columns and sediments in all locations were oligotrophic, according to Mediterranean values (suspended and sedimentary chlorophyll *a*: ~1 µg l^{-1} and ~1.2 µg g^{-1}, respectively) (Sarà et al. 1998, Sarà et al. 1999, CEOM 2002).

At each of the 3 fish farm locations, 6 sampling sites were positioned downstream along the main axis of the water current according to 3 distance categories (Fig. 1): (1) two sites were placed within a distance of 50 m downstream from the centre of the fish farm (hereafter referred to as 'cages' or 0 m sites), (2) two sites were placed ~500 m downstream (hereafter referred to as 500 m sites), and (3) the final 2 sites were placed at a distance of ~1000 m downstream from the centre of the fish farm (hereafter referred to as 1000 m sites). To compare results and to investigate the influence of natural variability on the cage systems, data were also collected at an external control location. At this control location off the northwest coast of Sicily (Egadi Island Strait), 6 sites were positioned at a depth of 15 to 20 m, each positioned along the main axis of the water current (mean velocity of current [MVC] ~20 cm s^{-1}), and at not more than 500 m from each other in order to simulate the same spatial pattern of the cage locations (Fig. 1). Inputs other than primary and terrigenous production were not present in this location (Sarà et al. 1999).

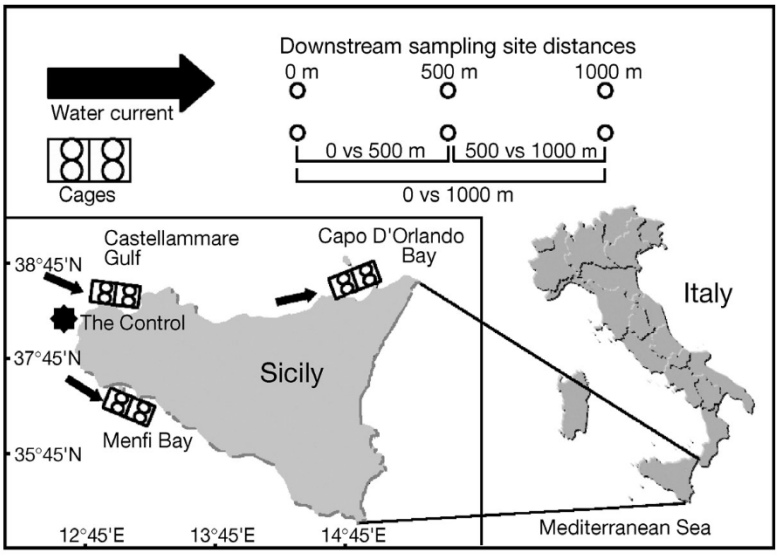


Fig. 1. Study areas (not to scale), including the control area (●: Egadi Islands). Scheme of fish farming systems also shown, depicting location of cages, distances of sampling sites from cages, type of comparisons among sites, and direction of main water current

Laboratory analysis. Water samples were collected at a depth of about 14 m (1 m below the end-edge of the cage net), while sediments were taken from 2 quadrats (400 cm^2 surface area) using hand cores (collected by SCUBA divers). In the laboratory, water samples (~4 l) were screened through a 200 µm mesh net in order to remove larger zooplankton and debris, and filtered through pre-combusted fibreglass filters (Whatman GF/F) (at 450°C for 4 h). The top layer (0 to 1.5 cm) of each core

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Table 1. Measurements of current velocity frequencies (%) within 5 cm s^{-1} increments up to 20 cm s^{-1} and higher in the study areas Gulf of Castellammare (HYDRO 1: mean velocity of current [MVC] = 12.0 ± 7.5 cm s^{-1}), Menfi Bay (HYDRO 2: MVC = 21.0 ± 11.8 cm s^{-1}), and Capo D'Orlando Bay (HYDRO 3: MVC = 41.4 ± 18.4 cm s^{-1})

	Current velocity increments (cm s^{-1})			
	5	10	15	>20
HYDRO 1				
Subsurface (3 m)	23	44	25	8
Mean depth (10 m)	22	27	23	27
Bottom (30 m)	20	24	23	33
HYDRO 2				
Subsurface (3 m)	40	30	0	30
Mean depth (10 m)	50	30	0	20
Mean depth (20 m)	43	37	0	20
Bottom (30 m)	33	0	0	66
HYDRO 3				
Subsurface (3 m)	14	26	17	44
Mean depth (10 m)	5	24	22	49
Mean depth (20 m)	4	17	19	60
Bottom (30 m)	4	8	14	74

was immediately frozen at –20°C and stored until analysis. About 10 specimens of cultivated fish were sampled and muscle tissue dissected and, after an overnight evacuation, fish faeces were collected using pipettes. In addition, samples of pelletted foods used in the fish farms were collected for carbon and nitrogen isotopic analysis. Water (POM) and sediments (SOM) as well as muscles, faeces and pellets were analysed to measure carbon and nitrogen isotope ratios. All samples were acidified in 2 N HCl, dried at 60°C for at least 24 h, then ground. Isotopic analyses were performed using a Finnigan Delta-Plus isotope ratio mass spectrometer. Isotopic values were expressed in parts per thousand as well as deviations from standards (Peedee belemnite limestone for $\delta^{13}C$, and nitrogen in air for $\delta^{15}N$):

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard})] \times 10^3$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Sampling design and statistical analyses. The control-impact sampling design enabled comparisons between putatively impacted and control locations over different distances. We used a series of partial (0 vs. 500 m; 0 vs. 1000 m; 500 vs. 1000 m) 3-way ANOVAs to evaluate the hypothesis that $\delta^{13}C$ and $\delta^{15}N$ of POM and SOM varied as a function of distance from the cages. The 1st factor (hydrodynamics: HYDRO) was treated as fixed and orthogonal. The 3 levels of hydrodynamics were low as measured in the Gulf of Castellammare (MVC ~12 cm s^{-1}: HYDRO 1), intermediate as measured in Menfi Bay (MVC ~22 cm s^{-1}: HYDRO 2), and high as measured in Capo d'Orlando Bay (MVC ~40 cm s^{-1}: HYDRO 3). The control location (Egadi Islands) was considered in the ANOVA as the 4th level of the HYDRO factor, with a MVC of ~20 cm s^{-1} (Lazzari et al. 1994). Distance was the 2nd factor in the ANOVA (3 levels: DISTANCE). Distance was treated as fixed and orthogonal. Sites (2 for each distance) were treated as random (2 levels: SITE) and nested in the interaction of the above factors. Three replicates were randomly collected at each site. Heterogeneity of variances was tested using Cochran's *C*-test, and appropriate means were compared using Student-Newman-Keuls (SNK) test (Underwood 1997).

We used the mixing model approach to infer the most important carbon and nitrogen sources qualitatively responsible for the isotopic composition of POM and SOM (Phillips & Gregg 2003, Sarà et al. 2003). We designated POM and SOM carbon and nitrogen signals (as measured throughout the study locations) as targets in the models. The end-members in the models were pelletted food (PEL: $\delta^{13}C$ = –22.6‰; $\delta^{15}N$ = 7.8‰) and fish faeces signals (EJE: $\delta^{13}C$ = –23.1‰; $\delta^{15}N$ = 10.7‰) as measured throughout the study locations. The phytoplankton (PHY: $\delta^{13}C$ = 19.0 ‰; $\delta^{15}N$ = 6.5 ‰), sand microflora (SM: $\delta^{13}C$ = –15.0 ‰; $\delta^{15}N$ = 6.5 ‰) and terrestrial signals (TER: $\delta^{13}C$ = –26.4 ‰; $\delta^{15}N$ = 1.9‰) were extrapolated from the literature (Dauby 1989, Martinotti et al. 1997, Riera 1997, Middelburg & Nieuwenhuize 1998, Mazzola & Sarà 2001, Sarà et al. 2003, Sarà et al. 2004).

Differences in percent contribution of each carbon and nitrogen source (terrigenous, primary [e.g. phytoplankton or sand microflora], and fish farm waste) at each combination of site and hydrodynamic level were analysed by cluster analyses (CA). Bray-Curtis similarity was used as a distance index. SIMPER procedure (cut-off: 100%) was employed to determine the organic matter source, which contributed the most to observed dissimilarities (Clarke 1993). Data were square-root transformed. The GMAV 5.0 statistical package (University of Sydney) and the PRIMER package (Plymouth Marine Laboratory) were used to perform uni- and multivariate analyses. The ISOSOURCE (Phillips & Gregg 2003) software package was used to run mixing equations.

RESULTS

Mean, minimum and maximum values of $\delta^{13}C$ and $\delta^{15}N$ in POM and SOM, recorded within each distance category at each location (including control sites located at the Egadi Islands), are shown in Table 2.

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Table 2. Measurements of $\delta^{13}C$ and $\delta^{15}N$ in particulate and sedimentary organic matter (POM and SOM, respectively) at each location in each distance category. CAST: Gulf of Castellammare (isotopic data from this site partially derived from Sarà et al. 2004); MEN: Menfi Bay; CORL: Capo D'Orlando Bay; EI: Egadi Islands (control sites)

Hydrodynamic level	Distance (m)	POM $\delta^{13}C$ Mean	(±SE)	Min.	Max.	SOM $\delta^{13}C$ Mean	(±SE)	Min.	Max.	POM $\delta^{15}N$ Mean	(±SE)	Min.	Max.	SOM $\delta^{15}N$ Mean	(±SE)	Min.	Max.
HYDRO 1 (CAST)	0	–22.3	(0.5)	–22.9	–21.9	–22.0	(0.1)	–22.1	–21.9	5.2	(0.2)	5.0	5.4	4.5	(0.1)	4.4	4.6
	500	–24.0	(0.4)	–24.5	–23.6	–22.1	(0.1)	–22.2	–22.0	2.9	(0.2)	2.6	3.0	3.1	(0.1)	3.0	3.2
	1000	–23.0	(0.1)	–23.1	–22.9	–22.2	(0.3)	–22.4	–21.8	3.5	(0.1)	3.4	3.6	2.3	(0.1)	2.2	2.4
HYDRO 2 (MEN)	0	–22.1	(0.2)	–22.3	–21.8	–20.2	(0.3)	–20.5	–19.9	2.6	(0.1)	2.5	2.6	4.2	(0.1)	4.1	4.2
	500	–22.0	(0.2)	–22.2	–21.8	–21.3	(0.4)	–21.9	–21.0	3.9	(0.1)	3.7	4.0	4.5	(0.1)	4.3	4.6
	1000	–22.4	(0.5)	–22.9	–21.9	–23.6	(0.2)	–23.8	–23.4	3.3	(0.1)	3.2	3.4	4.1	(0.1)	3.9	4.2
HYDRO 3 (CORL)	0	–22.0	(0.1)	–22.1	–21.8	–21.3	(0.2)	–21.4	–21.0	1.8	(0.1)	1.6	1.9	2.1	(0.2)	1.8	2.3
	500	–21.5	(0.1)	–21.6	–21.3	–23.7	(0.2)	–23.8	–23.4	4.2	(0.1)	4.1	4.4	3.4	(0.2)	3.2	3.6
	1000	–21.9	(0.2)	–22.1	–21.8	–22.5	(0.1)	–22.6	–22.4	4.6	(0.1)	4.5	4.6	4.4	(0.2)	4.1	4.6
Control (EI)	0	–22.7	(0.2)	–23.1	–22.2	–18.7	(0.2)	–19.3	–18.4	5.9	(0.3)	5.1	6.6	4.2	(0.2)	3.9	4.7
	500	–21.7	(0.6)	–23.2	–20.5	–18.3	(0.0)	–18.4	–18.2	5.0	(0.2)	4.6	5.4	4.4	(0.1)	4.1	4.6
	1000	–22.5	(0.1)	–22.5	–22.3	–17.9	(0.5)	–18.7	–16.8	5.3	(0.1)	5.1	5.6	4.6	(0.0)	4.5	4.6

C and N in POM and SOM at variable distances from fish farms

HYDRO 1—Gulf of Castellammare

Partial comparisons in the Gulf of Castellammare (i.e. at current velocity of 10 to 12 cm s^{-1}) showed significant differences (ANOVA, $p < 0.05$) in $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ values between the following sites: 0 and 500 m, 500 and 1000 m, and 0 and 1000 m (Fig. 2a). When moving from 0 m sites (pellet + ejection = 37%) to 1000 m sites (pellet + ejection = 13%), mixing model results showed a decreasing contribution of fish farm waste signals to POM. SOM results were similar to those of POM regarding nitrogen (Fig. 3a). There were significant differences among all distance categories (ANOVA, $p < 0.05$), which described a well-defined nitrogen-depletion gradient moving away from the focus of emission to farther sites. In contrast, when all distances were compared, $\delta^{13}C_{SOM}$ values were similar (ANOVA, $p > 0.05$). However, the contribution of total fish farm waste to carbon decreased with increasing distance from the centre of the fish farm (pellet + ejection = 31 and 3.3% at 0 and 1000 m sites, respectively).

HYDRO 2—Menfi Bay

In Menfi Bay, $\delta^{13}C_{POM}$ values did not show significant differences among the 3 distance categories (ANOVA, $p > 0.05$) (Fig. 2b) but remained notably constant up to 1000 m from the cages. In contrast, $\delta^{15}N_{POM}$ values were significantly different among all distance categories (ANOVA, $p < 0.05$), and described a nitrogen-enrichment gradient with increasing distance from the cages to far sites. With regard to POM, the decreasing contribution of total fish farm waste with increasing distance from the cages (pellet + ejection = 16%) to farthest sites (pellet + ejection = 1.5%) was clearly evident. In contrast, information from partial comparisons of SOM between distance categories indicated that $\delta^{13}C_{SOM}$ values showed a significant depletion gradient with increasing distance from cages (ANOVA, $p < 0.05$), whereas $\delta^{15}N_{SOM}$ appeared almost constant between the cages and farthest sites (Fig. 3b). The trend of the percent contribution of total fish farm waste to sediments showed an inverse gradient with respect to the water column: this contribution was lower at cages (pellet + ejection = 7.5%) and higher at 1000 m sites (pellet + ejection = 38%).

HYDRO 3—Capo D'Orlando Bay

In Capo D'Orlando Bay, $\delta^{13}C_{POM}$ values were similar among all distance categories (ANOVA, $p > 0.05$), whereas $\delta^{15}N_{POM}$ showed a clear and significant enrichment gradient (ANOVA, $p < 0.05$) with increasing distance from the cages ($\delta^{15}N_{POM}$ = 1.8‰) toward more distant sites ($\delta^{15}N_{POM}$ = 4.6‰) (Fig. 2c). Thus, the total contribution of fish farm waste to POM increased significantly with increasing distance from cages (pellet + ejection < 1%) to 1000 m sites, where highest values were detected (pellet + ejection = 19%). For SOM, all distance categories were significantly different in both $\delta^{13}C_{SOM}$ and $\delta^{15}N_{SOM}$ composition (ANOVA, $p < 0.05$) (Fig. 3c); values were greater at more distant sites compared to close sites, and thus depicted a well-defined

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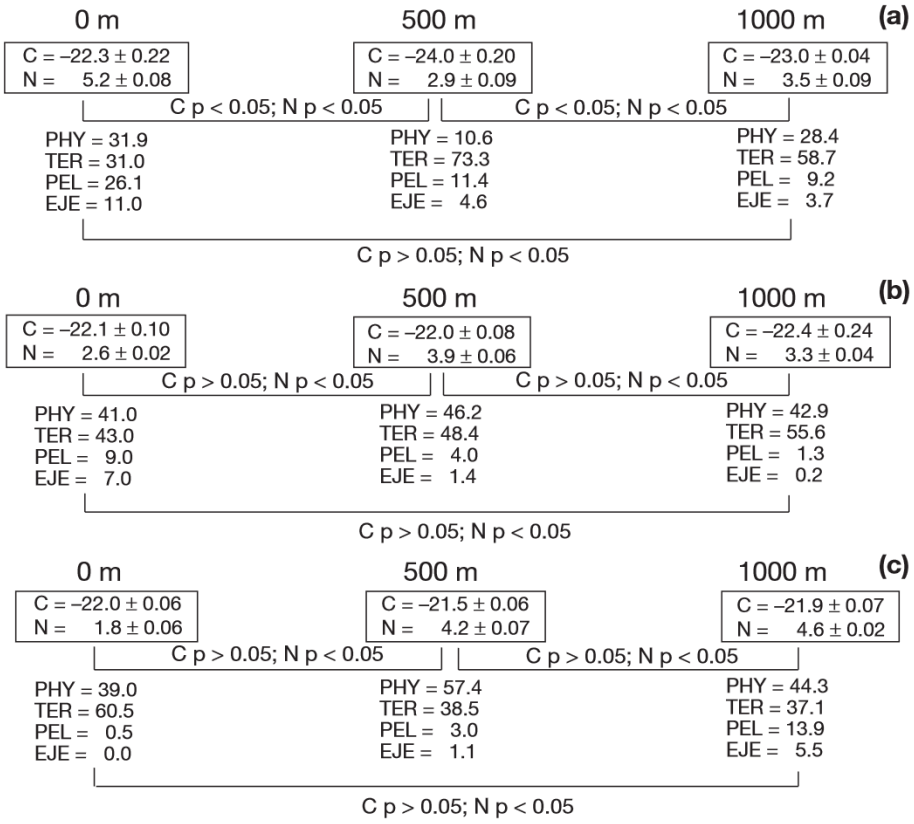


Fig. 2. Particulate organic matter (POM) mean isotopic values (‰) ±SE of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measured at each distance; significant differences between distance categories (0 vs. 500 m; 500 vs. 1000 m; 0 vs. 1000 m) indicated by $p < 0.05$ (ANOVA: df = 12, all ANOVA assumptions met). (a) Gulf of Castellammare (HYDRO 1); (b) Menfi Bay (HYDRO 2); (c) Capo D'Orlando Bay (HYDRO 3). Percent contribution of each POM source also reported—PHY: phytoplankton contribution; TER: terrigenous-continental contribution; PEL: pellet contribution; EJE: ejection from fish contribution

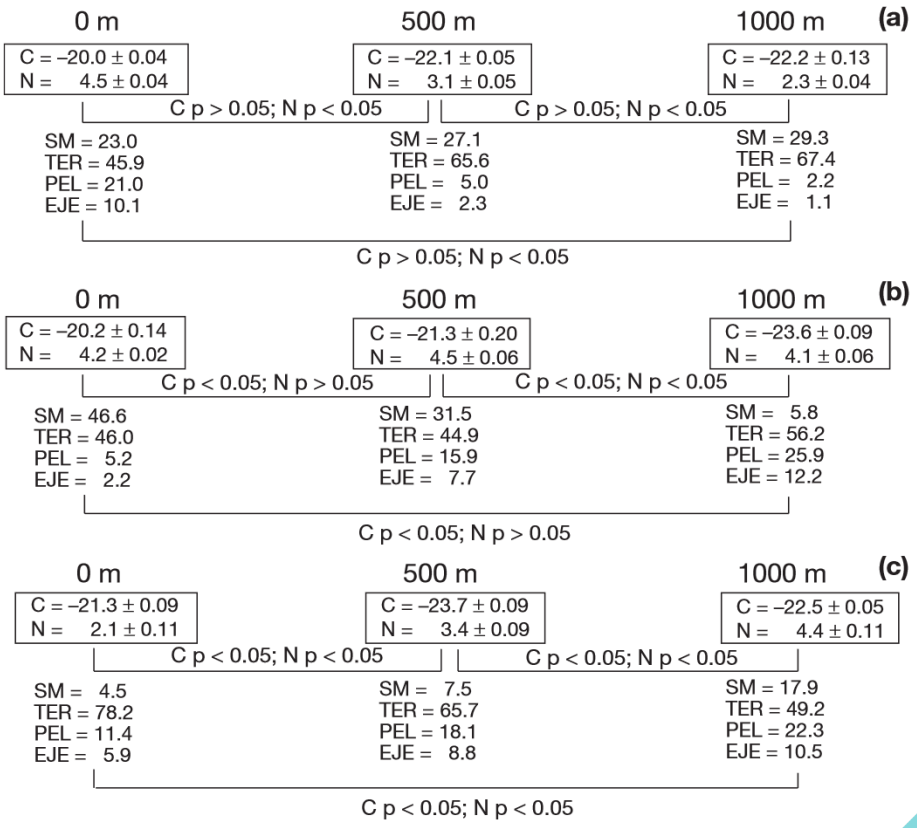


Fig. 3. Sedimentary organic matter (SOM) mean isotopic values (‰) ±SE of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measured at each distance. Percent contribution of each SOM source also reported—SM: sand microflora contribution. See Fig. 2 for further details

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enrichment gradient. The total contribution of fish farm waste to SOM also increased with increasing distance from cages (pellet + ejection = 17 %) to more distant sites (pellet + ejection = 33 %).

HYDRO Control—Egadi Islands

In the Egadi Islands (control location), $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ values showed significant differences (ANOVA, $p < 0.05$) among the 3 distance categories, except for N between the 500 and 1000 m distances (Fig. 4a). In contrast, the isotopic composition of SOM was almost constant among distance categories, and did not show significant differences (ANOVA, $p > 0.05$) for either $\delta^{13}C$ or $\delta^{15}N$ values (Fig. 4b). The mixing model run with only 1 mixture and 2 sources indicated that the overall terrigenous contribution ranged on average between 23 and 41 %, whereas the autochthonous contribution (phytoplankton and sand microflora for POM and SOM, respectively) ranged on average between 59 and 69 %.

Comparison of $\delta^{13}C$ and $\delta^{15}N$ in POM and SOM within distance categories

The comparison of $\delta^{13}C_{POM}$ values from 0 m sites among locations showed no significant differences (ANOVA, $p > 0.05$), while values from the 500 and 1000 m sites were considerably more variable than those from the 0 m sites, in particular because of the Gulf of Castellammore values (Fig. 5a). The origin of $\delta^{13}C_{SOM}$ was markedly different (ANOVA, $p < 0.05$) (Fig. 5b); while no clear trends were apparent with regard to location, values were significantly different among all locations for all 3 distance categories. For $\delta^{15}N_{POM}$ (Fig. 5c), each location was significantly different from the others within each distance (ANOVA, $p < 0.05$), whereas $\delta^{15}N_{SOM}$ values at Capo D'Orlando Bay (CORL) and Gulf of Casetellammare (CAST) were significantly different from each other (ANOVA, $p < 0.05$) only at the 0 and 1000 m sites, respectively (Fig. 5d).

All systems together

The cluster analysis (Fig. 6) performed on the POM mixing model percent data (converted to a similarity matrix using the Bray-Curtis similarity coefficient) isolated 3 clusters: a first cluster with control sites from Egadi Islands (EI) grouping close together; a second cluster (hereafter referred to as cluster POM A) grouping Menfi Bay (MEN) HYDRO 2 (500 m), MEN HYDRO 2 (1000 m), CORL HYDRO 3 (0 m) and CORL HYDRO 3 (500 m) with an average Bray Curtis similarity of 87 %; and a third group (hereafter referred as cluster POM B) grouping CAST HYDRO 1 (0 m), CAST HYDRO 1 (500 m), CAST HYDRO 1 (1000 m), MEN HYDRO 2 (0 m) and MEN HYDRO 3 (1000 m). The highest level of average Bray-Curtis dissimilarity was recorded for comparisons between EI control group sites and sites belonging to POM B group. The POM A group exhibited an intermediate position (Fig. 6). In all comparisons, the sources contributing most to dissimilarities were phytoplankton contribution (PHY) and pelletted food (PEL) (Fig. 6).

The cluster analysis (Fig. 7) performed on SOM mixing model percent data identified 3 main Bray-Curtis groups. The first group (hereafter referred to as CTRL) represented all control sites. The second group (here-

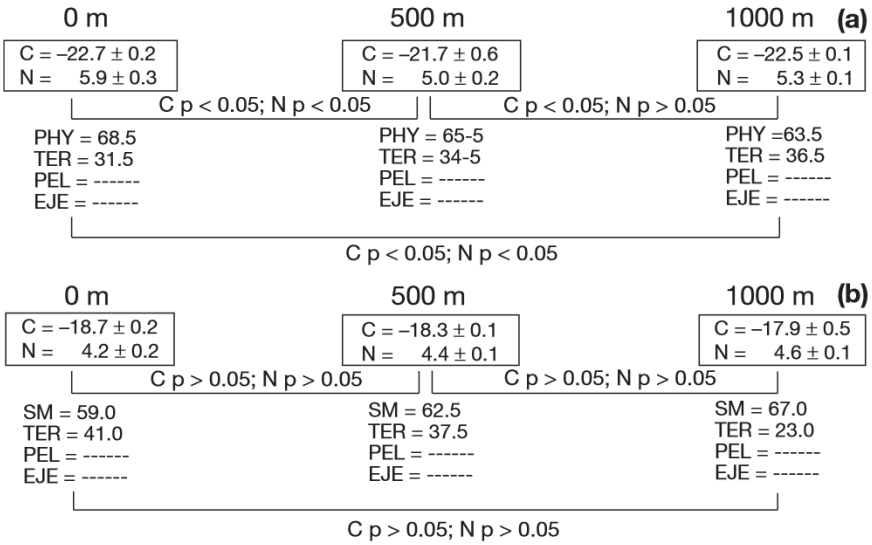


Fig. 4. Mean isotopic values (‰) ±SE of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measured at each distance at Egadi Islands. Significant differences between distance categories (0 vs. 500 m; 500 vs. 1000 m; 0 vs. 1000 m) indicated by $p < 0.05$ (ANOVA). Percent contribution of each organic matter source also reported for (a) POM and (b) SOM; see Figs. 2 & 3 for abbreviations

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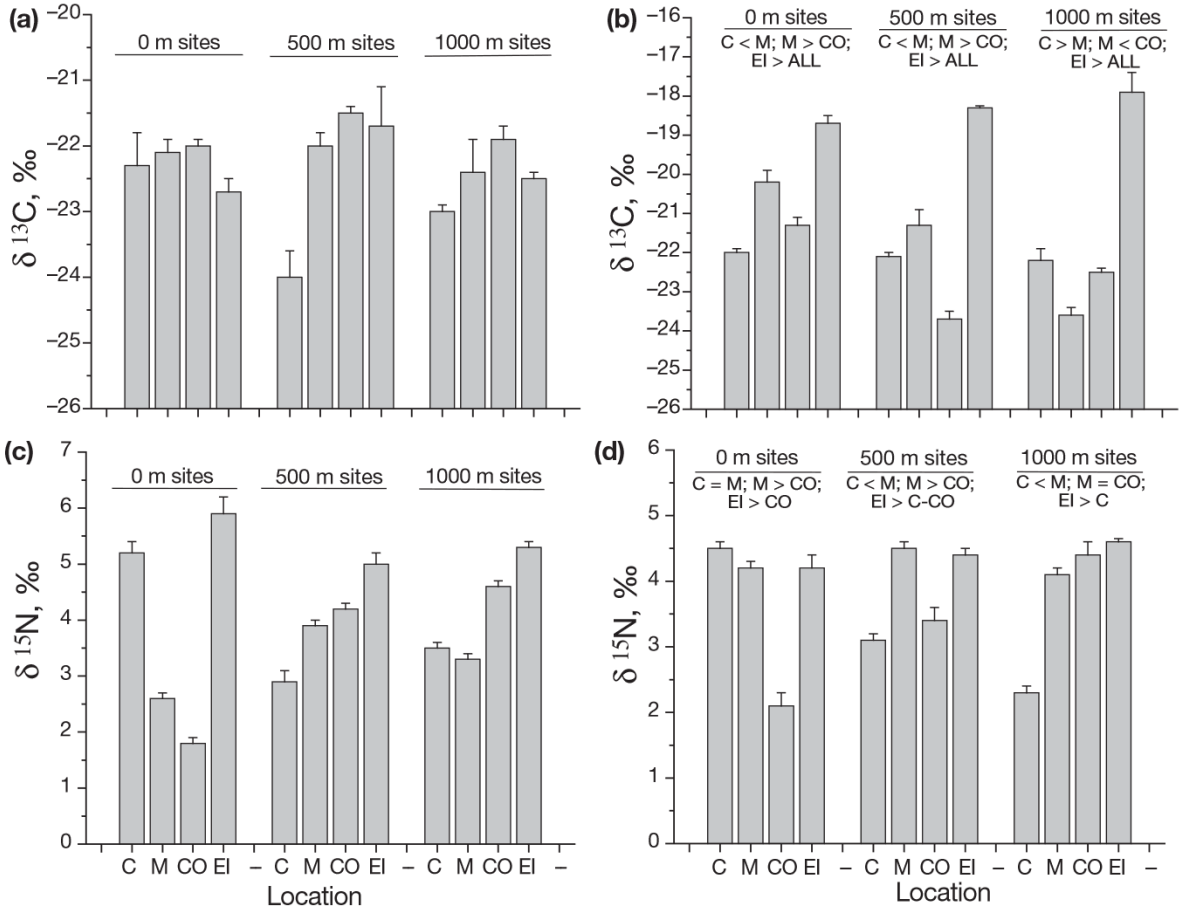


Fig. 5. Mean (±SE) isotopic values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for distance categories and locations. (a) POM $\delta^{13}C$; (b) SOM $\delta^{13}C$; (c) POM $\delta^{15}N$; (d) SOM $\delta^{15}N$. Comparisons using Student-Newman-Keuls tests reported inside SOM graphs. C = Gulf of Castellammare; M = Menfi Bay; CO = Capo d'Orlando; EI = Egadi Islands (control)

after referred to as SOM A) represented 3 sites, which could be categorized as pristine sites in the cage areas: CAST HYDRO 1 (500 m), CAST HYDRO 1 (1000 m), and MEN HYDRO 2 (0 m). The third group included the impacted locations (hereafter referred as SOM B), which were further grouped into 2 subgroups: a low impact subgroup represented by MEN HYDRO 2 (500 m), MEN HYDRO 2 (1000 m) and CORL HYDRO 3 (0 m); and a high impact subgroup that included CAST HYDRO 1 (0 m), MEN HYDRO 2 (500 m), and CORL HYDRO 3 (1000 m). The highest level of average dissimilarity was recorded in the pair-wise comparison of CTRL sites with SOM B sites; SOM A exhibited an intermediate level of Bray-Curtis dissimilarity (Fig. 7). In all comparisons, the sources contributing most to dissimilarities were SM and PEL (Fig. 7).

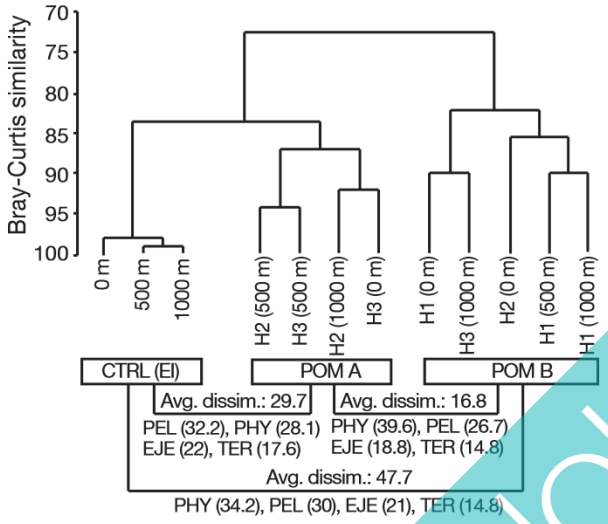


Fig. 6. Cluster analysis of POM mixing model percent data (Bray-Curtis similarity index). Average dissimilarity (Avg. dissim., %) between groups, and results of SIMPER procedure for contribution of each POM source (TER, PEL, EJE, PHY; cut off: 100%), also reported. EI: Egadi Islands (control). For acronyms, see Figs. 2 & 3

DISCUSSION

Our study highlighted that the effect of waste from fish farms on the area surrounding fish cages was a

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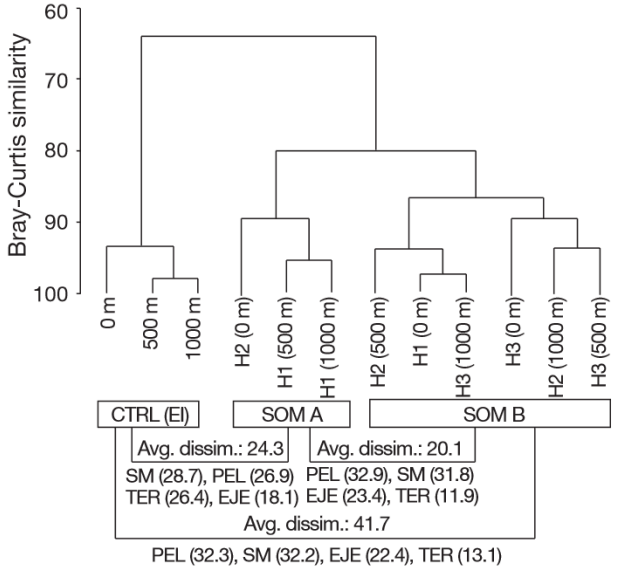


Fig. 7. Cluster analysis of SOM mixing model percent data (Bray-Curtis similarity index). See Fig. 6 for further (equivalent) details; Fig. 2 for acronyms

function of different levels of hydrodynamics. Other researchers, with similar objectives, have consistently emphasized the importance of hydrography on a local scale (Wu et al. 1994, Wu 1995). Nevertheless, previous studies have never before shown a clear effect of waste products beyond 100 to 300 m from the centre of cage emissions (Pearson & Black 2000). In contrast, by using the stable isotope approach, our results enabled us to trace organic enrichment over larger distances. Our 3 study locations were characterised by remarkably different current velocities, and the resulting hydrodynamic patterns explained the different influences of fish farm waste on the surrounding environments of each location.

In the Gulf of Castellammare, low current velocities produced deposits of cage waste at a distance less than 100 m from the point-source. When moving away from fish farm cages, the isotopic contribution of cage waste quickly decreased, and it contributed less than 10% to total POM and SOM at the farthest sites.

When the current velocity approached about 20 to 22 cm s^{-1}, as in Menfi Bay, the fish farm waste contribution decreased in the water column (not more than 10 to 15%) with increasing distance from the cages; as was also observed at the Gulf of Castellammare location, peak contributions were apparent close to the cages. In contrast, the SOM exhibited an inverted pattern; cage waste contributions to SOM increased linearly with distance. Waste contributions reached a peak at the 1000 m distance, where it comprised about 38% of total SOM.

In Capo D'Orlando Bay, where the MVC was high, the percent contribution of fish farm waste organic matter increased with increasing distance from the cages. Waste particles were influenced by a 'sail effect', due to high currents that dispersed waste particles to farthest distances and reduced isotopic traces at sites closer to the cages. In the sediments, some particles settled near the cages (about 16% of the total), whereas most settled at greater distances, contributing about 28% and 34% to total SOM at 500 m and 1000 m, respectively. Recent studies by other researchers supports our results. Using manipulative experiments, Cromey et al. (2002) modelled the 'rolling' activity of waste particles under the influence of bottom water currents. Artificial particles that simulated the specific weight and size of common salmon feed were moved several hundreds of meters from cages in the presence of high velocity currents at the bottom of the water column. This agrees with the idea that resuspension movements from a bottom current can be a major factor that influences the extension plume from point-source disturbance. In addition, our results suggested that under a given situation (depth of ~25 to 30 m and current velocity of ~18 to 40 cm s^{-1}), sediment resuspension can enhance the effects of fish farm waste (in the form of pellets and ejections) by enlarging its spatial influence by about 10 to 20 times that observed at locations with lower current velocities (value extrapolated from the difference between the extension area at Gulf of Castellammare, Menfi Bay, and Capo d'Orlando). Cluster analyses provided important confirmation regarding the variable impact of waste from fish farms depending on distance and current velocity.

The water column and sediments appear to react differently to fish farm waste when influenced by different hydrodynamic levels. In the last 2 decades, most studies have focused on water column dynamics in order to detect impacts of fish farming activities. Some have analysed the dynamics of the dissolved nutrient pool or the features of suspended organic matter (La Rosa et al. 2002, Alongi et al. 2003) and the free living and attached fraction of suspended bacteria or phytoplankton (McKinnon et al. 2002). More recently, other authors have considered sediment dynamics in their protocols, and have determined that the overall features of SOM result in an underestimation of the real importance of the role of this bulk (sensu Danovaro et al. 2003). Nevertheless, our isotopic approach allowed us to infer that the water column represented an aleatory subsystem with respect to the sediments. At the control location (Egadi Islands), the isotopic composition of the water column was naturally variable and showed overall differences in both $\delta^{13}C$ and $\delta^{15}N$ among sites separated by a distance of 500 m. Such dif-

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ferences were not observed in sediments, which in the absence of anthropogenic inputs were similar in composition among sites positioned 500 m apart. Thus, by only studying variability in the water column, the extrapolation of information on the effect of fish farm waste on the marine environment (and perhaps on other impacted aquatic systems) might be biased; sediments may often provide more reliable interpretations.

CONCLUSIONS

We detected some effects of organic loading from fish farm waste on the surrounding environment at distances up to 1000 m from the cages under variable environmental conditions. Nevertheless, we did not detect any 'impact' that implied biological consequences and environmental costs. Since we analysed a chronic impact at each location (off-shore fish farming is not seasonal), it is likely that organic enrichments observed at different distances may lead to structural rearrangements of benthic assemblages. However, we only detected a deviation from natural organic enrichment. Pitta et al. (1999) stressed the need for reconsideration of the 'impact' concept and spatial influence in future investigations. Vivificated oligotrophic waters from our study locations seemed to be able to accommodate organic waste (more than 40 to 50 tons) originating from fish farms, converting it into fresh biomass and still maintaining the system under the impact threshold.

Therefore, researchers should reconsider the role of the effects from offshore fish farms according to the following points: (1) the relative area of influence of the impacts of fish farms seems to proportionally increase with increasing current velocities; (2) the water column appears to be an 'aleatory' mirror of the trophic situation, whereas sediments appear to mirror the real dispersion; and lastly (3) the distribution of waste from the cages seems to be dependent on movements at the bottom of the water column, which confirms the recently identified role played by resuspension movements (Cromey et al. 2002).

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