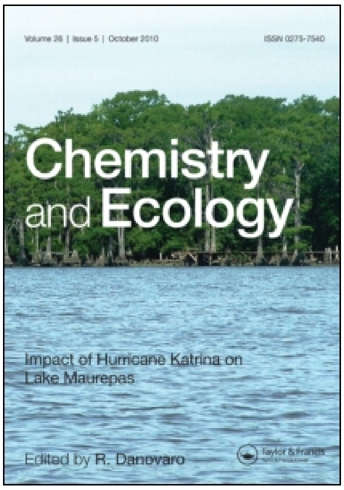

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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Online publication date: 14 September 2010

To cite this Article Danovaro, R. , Corinaldesi, C. , La Rosa, T. , Luna, G. M. , Mazzola, A. , Mirto, S. , Vezzulli, L. and Fabiano, M.(2003) 'Aquaculture impact on benthic microbes and organic matter cycling in coastal mediterranean sediments: A synthesis', Chemistry and Ecology, 19: 1, 59 — 65
To link to this Article: DOI: 10.1080/0275754031000084392
URL: http://dx.doi.org/10.1080/0275754031000084392

Chemistry and Ecology, 2003, Vol. 19(1), pp. 59–65

AQUACULTURE IMPACT ON BENTHIC MICROBES AND ORGANIC MATTER CYCLING IN COASTAL MEDITERRANEAN SEDIMENTS: A SYNTHESIS

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(In final form 7 January 2003)

Microbial assemblages and organic matter composition as well as their response to the disturbance induced by mussel and fish-farm biodeposition were compared in several areas of the Mediterranean: La Spezia (Ligurian Sea), Gaeta (Tyrrhenian Sea), Cattolica (Adriatic Sea) and Cyprus (Levantine Sea), on both unvegetated and *Posidonia* bed sediments. In all systems investigated, organic matter (as biopolymeric carbon) accumulated in aquaculture impacted sediments. Among the main biochemical classes, lipids appeared to be a good tracer of aquaculture impact, especially in fish-farm sediments. Exoenzymatic activities displayed higher values in sediment beneath the cages, indicating faster organic matter cycling. A significant accumulation of chloroplastic pigments was observed under mussel farms (related to the deposition of faeces and pseudo faeces), but also on fish-farm sediments. The presence of newly deployed cages induced rapid changes in the benthic compartment, and sediments reached rapidly (6 weeks) reducing conditions. Bacterial and total microbial density increased significantly in impacted sediments. Although the contribution of prokaryotic auto-fluorescent cells was very low, this component displayed a clear response to organic biodeposition, increasing significantly beneath the cages. Conversely, eukaryotic auto-fluorescent cells decreased. The ratio of benthic auto-fluorescent cells to total bacterial number proved to be a new, sensitive descriptor of biodeposition impact. Heterotrophic bacteria (CFU, and particularly the number of gram-negative bacteria: *Cytophaga*/*Flexibacter*-like bacteria) increased significantly in sediments beneath the farm. The similarities and differences observed among sites, latitudes, microbial components and typology of impact (mussel *vs.* fish farm) are analysed and discussed.

Keywords: Benthic microbes; Mussel and fish-farm impact; Biodeposition; Sedimentary organic matter

1 INTRODUCTION

In the Mediterranean Sea aquaculture activities have expanded significantly during last years, inducing an increasing interest and concern for their potential impact on coastal marine environments (Rana, 1998). The most evident effects of the fish cages on bottom sediments are the accumulation of organic matter and the progressive transformation of the bottom

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ISSN 0275-7540 print; ISSN 1029-0370 online
DOI: 10.1080/0275754031000084392

sediments into a flocculent anoxic surface (Holmer, 1991; Holmer and Kristensen, 1992; Karakassis *et al.*, 1998). Mussel farm impact on benthic environment is likely to be different from fish-farming impact (Mirto *et al.*, 1999a). The biodeposition of faeces and pseudo-faeces due to mussel farming might cause modifications, though generally of limited extension, of the physical and chemical features in the sediment beneath the long-lines (Dahlbäck and Gunnarsson, 1981; Gilbert *et al.*, 1997; Kaspar *et al.*, 1985) able, nonetheless, to affect benthic community structure (Castel *et al.*, 1989; Dinet *et al.*, 1990; Kröncke, 1996; Mazzola *et al.*, 2000) and organic matter cycling and composition (Dahlbäck and Gunnarson, 1981; Kaspar *et al.*, 1985; Barranguet *et al.*, 1993). The organic load due to the fish-farm activity, which employ external sources of organic matter (*i.e.*, food pellets), might result in highly eutrophic conditions, determining reducing conditions, long-term changes in the structure of the benthic assemblages (reduced density and biodiversity) and increasing the importance of the smaller food web components (*i.e.*, bacteria; La Rosa *et al.*, 2001a; Danovaro *et al.*, 1999; Brown *et al.*, 1987; Frid and Mercer, 1989; Weston, 1990; Holmer, 1991; Hansen and Blackburn, 1992; Mirto *et al.*, 1999b; Mirto *et al.*, 2000). Despite the increasing interest for the effects of aquaculture on coastal environments, studies on the benthic impact due to mussel and fish-farming are still scarce as far as the microbial component is concerned (Mattson and Linden, 1983; Radziejewska, 1986; Dinet *et al.*, 1990; Grenz *et al.*, 1990; Barranguet *et al.*, 1993; Wu, 1995).

The structure of microbial assemblage is sensitive to changes in environmental conditions and trophic state (Danovaro, 2000; La Rosa *et al.*, 2002), especially when subjected to nutrient input related to anthropogenic activity (Jensen *et al.*, 1990; Hansen and Blackburn, 1992). For these reasons and for their role in biogeochemical cycles, measurement of microbial assemblages have been recently proposed as tools for monitoring the impact of organic enrichment following intensive aquaculture activities (La Rosa *et al.*, 2001a; Vezzulli *et al.*, 2001).

This study was designed to investigate, at a comparative level, the effects of mussel- and fish-farms on the coastal benthos, by means of microbial indicators. The microbial response to farm biodeposition was investigated in different Mediterranean areas (Ligurian, Tyrrhenian, Adriatic and Levantine Seas) to gather information of consistency and comparability of altered processes identified. Initial and long-term effects of fish farming on benthic microbial assemblages were also investigated to provide management tools for aquaculture development. Microbial indicators of aquaculture impact are proposed for future biomonitoring studies aimed at preserving the sustainability of aquaculture activities.

2 MATERIALS AND METHODS

2.1 Study Areas and Sampling

This study compares the results of investigations carried out at two mussel farms located in the Adriatic Sea (Cattolica) and in the Tyrrhenian Sea (Gaeta) and at three fish farms located in the Ligurian Sea (La Spezia), Tyrrhenian Sea (Gaeta) and Levantine Sea (Cyprus). The mussel farm located in Cattolica (Adriatic Sea at 11-m depth) was characterised by sandy sediments. Farm extension was ca 2 km^2 and produced ca 1000 tons per year. Long lines were placed at ca 40–50 m distance each other. The fish farm of Gaeta (Tyrrhenian Sea) was investigated from March 1997 to October 1997. The area is characterised by limited seasonal changes in temperature and by the presence of two major rivers: the Garigliano and the Volturno. Dominant currents flow in SE–NW direction. The study area is sheltered and displays sandy-mud sediments. *Posidonia oceanica* meadows are present in the northern part. The fish farm (Farmocean, 2000 m^3) contained about 120,000 specimens of *Dicentrarchus labrax*

(18 kg m^3). The first sampling was carried out two weeks after cage deployment. In July 1997 the fish farm was removed from the area. This allowed evaluating changes in the benthic environment during recovery. The fish farm located in the Ligurian Sea (La Spezia, 15 years old) is located in a semi-enclosed bay and was investigated between June 2000 and February 2001. The study area is sheltered (water depth 10 m) and displays muddy sediment. Dominant currents flow in the SE–NW direction. The farm consisted of a total of 88 cages (each 36 m^3) and contained a biomass of 16 fish tons. The fish farm located in the Levantine Sea (Cyprus) was investigated in June 2002 at depths ranging from 35 to 40 m and contained both *Dicentrarchus labrax* and *Sparus aurata.* Sandy-mud sediments characterised the area covered by *Posidonia oceanica* meadows. At all sites a preliminary survey was carried out to determine the spatial extent of the aquaculture impact and for identifying the Control stations. Sediment samples (top 2-cm) were collected manually by SCUBA at impacted and control stations located at the same depth (from 10 to 38 m depending on farm location).

2.2 Environmental Parameters

Redox potential discontinuity (RPD) depth was estimated at the depth at which sediment colour turns from brown to black. Parallel Eh measurement indicated that this depth corresponded to the presence of sub-oxic conditions ($<$200 mV). Organic matter content and composition were determined according to Fabiano and Danovaro (1994), and reported as biopolymeric carbon content (BPC). Lipids were determined according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Protein analyses were carried out according to Hartree (1972). Carbohydrates were analysed according to Gerchacov and Hatcher (1972). For each analysis about 0.5 g of sediment was used. Analyses of sedimentary chlorophyll-a (Chl-a) and phaeopigments (Phaeo) were carried out according to Lorenzen and Jeffrey (1980). Data were normalised to dry weight (60 °C, 24 h) and expressed as $\mu gC\,g^{-1}$ of sediment.

2.3 Microbial Analyses

Bacterial cells were counted using epifluorescence microscopy after staining with DAPI, Acridine orange or SYBR Green. Pico-sized prokaryotic and eukaryotic cells were classified according to their autofluorescent spectrum: phycoerythrin-phycocyanin rich cyanobacteria (yellow–orange fluorescence) and chlorophyll-dominant (red–green fluorescence), respectively. Total bacteria and eukaryotic cell biomass was estimated by assigning cells to different size classes assuming 310 and 220 fgC μm^{-3}, respectively (Fry, 1990). Autotrophic bacterial density was converted into biomass assuming 294 fgC $cell^{-1}$. All data were normalised to dry weight and expressed as $\mu gC\,g^{-1}$. Plate counts of viable aerobic heterotrophic bacteria, expressed as colony-forming units (CFU) g^{-1}, were determined using Bacto marine agar 2216 (Difco) medium. Cellular morphology and presence of spores were determined using Gram stain procedure. Catalase test and fermentation/oxidation of glucose assay were performed in appropriate differential media. The search of halophilic vibrios was carried out on plates of TCBS (thiosulphate, citrate, bile and sucrose) agar. Total presumptive vibrios (PV) and presumptive *Vibrio parahaemolyticus* (PVP) were counted (Crisafi *et al.*, 1986). The quality of sediment was evaluated by *Escherichia coli* and *Enterococci* counts after incubation on C-EC Agar MUG (Biolife) and on Enterococcus Agar (Difco) plates. In order to confirm *E. coli* colonies, James' indole reagent (API bioMérieux) was used. Analyses of extracellular enzymatic activity (aminopeptidase, glucosidase and phosphatase) were carried out in triplicate by adding fluorogenic substrates (final concentration 200 μM), as described by Hoppe (1993).

3 RESULTS AND DISCUSSION

3.1 Fish- and Mussel-farm Impact on the Sediment Characteristics

Fish farming activities reduced oxygen penetration into the sediments beneath the cages. Reduced oxygen conditions were generally more evident in spring–summer as a result of the increased reared fish biomass, food input and bottom temperatures. Fish-farm impact resulted in the accumulation of organic matter in sediments underneath the fish cages (Mazzola *et al.*, 1999). In the Ligurian and Tyrrhenian Sea clear differences between fish farm and control sediments were observed in terms of biopolymeric C concentrations (Mirto *et al.*, 1999c; La Rosa *et al.*, 2001a; Vezzulli *et al.*, 2001). These data indicate that biopolymeric carbon concentration is a quite sensitive descriptor of the farm impact on the sediments. Also chlorophyll-a concentrations were significantly higher beneath fish cages. At all sites, lipid concentrations showed large differences between impacted and control stations. The very high lipid concentrations detected in fish-farm sediments appeared to be strictly related to the farming activity and can be used as an indicator of the organic input coming from the aquaculture activity. Also carbohydrate concentrations were significantly higher in sediments beneath the fish farms, whereas protein concentrations did not change significantly (Mirto *et al.*, 2000). Since protein input is likely to be larger under the farms, this may be attributed to the high protein turnover in farm sediments when compared to the control (as indicated by the much higher aminopeptidase activities in sediment beneath the cages). In general all enzymatic activities were significantly higher in impacted sediments, reaching at Cyprus values up to ten times higher than in the control. The deployment of a new fish farm in a non-impacted coastal area (Gaeta Gulf, Tyrrhenian Sea) induced rapid and evident changes in the sediment characteristics (Mazzola *et al.*, 1999). Reduced oxygen penetration in the sediment was observed after only 6 weeks. The initial impact resulted in the eutrophication of the farm sediments, evident in terms of phytopigment concentrations, followed by a decreased phytopigment content in summer as a result of the increased disturbance. Also mussel farms induced evident changes in the sediment characteristics. Oxygen penetration into the bottom sediments was significantly reduced in impacted stations, except in winter, as a result of the hydrodynamic forcing and sediment mixing. Biodeposition due to mussel-farm activities determined, in both the Tyrrhenian and the Adriatic Sea, a conspicuous increase of total phytopigment concentrations (chlorophyll-a concentrations up to $50\,\mu g\,g^{-1}$), with values significantly higher than in the Control. Mussel biodeposition was also detectable in terms of organic carbon concentrations, accumulation of proteins, lipids and, to a lesser extent, carbohydrates.

3.2 Fish- and Mussel-farm Impact on Microbial Assemblages

Bacteria displayed a clear response to increasing organic matter loads coming from fish farming. In farm sediments, bacterial and auto-fluorescent microbial biomass increased 3- and 10-folds, respectively. Microphytobenthic biomass in farm sediments was, on average, about double than in the Control, indicating that this component might be largely stimulated by fish-farm biodeposition. All microbial components appeared to be highly sensitive to fish-farm biodeposition, and 15 days after cages removal decreased to values indistinguishable from the Control. These results clearly indicate that bacterial biomass and particularly the auto-fluorescent microbial component are good descriptors of fish-farm disturbance. The ratio of auto-fluorescent cells to total bacterial abundance increased considerably in impacted stations (La Rosa *et al.*, 2001e). The majority of culturable bacteria were facultative anaerobes. Gram-negative bacteria constituted the predominant group in fish-farm sediments, whereas Gram-positive bacteria were prevalent (up to 90%) in the control site. Changes in the structure of microbial assemblages with the predominance of gram-negative bacteria in farm sediments could be attributed to the presence of antibiotic resistant strains in fish-farm impacted areas. Fermentative bacteria prevailed at fish-farm stations, confirming that anaerobic metabolism plays a major role in organic-rich sediments.

Evidence for a rapid impact of the seacages on benthic bacterial assemblages is provided by the increase of autofluorescent microbial cells (up to 11 times when compared to the Control). Conversely, eukaryotic cell contribution to total autofluorescent microbial density decreased significantly in impacted sediments. In impacted sediments of both Ligurian and Levantine Sea, the percentage of active bacteria was very low (Vezzulli *et al.*, 2001; Luna, pers. comm.) indicating that a large fraction of bacteria was dormant, dead or non-culturable. In farm sediments of the Ligurian and Tyrrhenian Sea, organic enrichment resulted in a significant increase of the number of viable heterotrophic bacteria. Bacteria belonging to the genus *Vibrio* increased in farm sediments of the Ligurian and Tyrrhenian Sea, but pathogenic bacteria did not change significantly. Viable faecal coliforms (as *E. coli* and Enterococci), investigated to assess the effects of others pollution sources (*i.e.* urban waste fall, terrigenous input) did not show differences between impacted and control areas.

In both Tyrrhenian and Adriatic sites interested to mussel farming, bacterial densities increased compared to the Control, but significant differences were observed only in periods of highest mussel production (December–February). The auto-fluorescent microbial density in mussel-impacted stations was 3 times higher than in the Control. Changes in the bacterial community structure beneath mussel farms can be summarised with a strong decrease of the picoeukariotic contribution to the total autofluorescent microbial density and an increase of cyanobacteria. Therefore mussel farms induced an increase of the procaryotic-autotrophic component and a decrease of the eukariotic fraction.

3.3 Fish-farm Impact on Different Benthic Components

In highly enriched environments, as those interested by fish-farm biodeposition, microbial components tend to increase, whereas metazoans (*e.g.*, meio and macrofauna) are reduced so that the ratio of bacterial to meiofaunal biomass increases (Danovaro *et al.*, 1999; Danovaro, 2000). The ratio of auto-fluorescent cells to total bacterial density increased in impacted sediments, but also the ratio of autofluorescent microbial biomass to meiofaunal biomass was about 8 times higher in farm sediments. The ratio of total bacterial to meiofaunal biomass was 3 to 4 times higher beneath the cage than in Control areas and the ratio of microphytobenthos to meiofauna biomass was about double in farm sediments. All these parameters recovered to undisturbed conditions immediately after cage removal, suggesting that the ratios between different microbial and metazoan components of benthic assemblages are sensitive descriptors of this kind of impact.

Acknowledgements

This work has been financially supported by MIPA-CNR (Italy) and by the EU-programme MEDVEG.

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