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Impact of aquaculture on benthic virus–prokaryote interactions in the Mediterranean Sea

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ABSTRACT

We investigated the effects of organic enrichment due to the biodeposition from fish farms on benthic prokaryotic and viral abundance and production, viral-induced prokaryotic mortality, enzymatic activities and bacterial diversity. We compared four areas across the Mediterranean Sea, from Cyprus to Spain, and two different habitats: sediments covered by the seagrass *Posidonia oceanica* and soft-bottom unvegetated sediments. In several cases, the sediments beneath the cages showed higher prokaryotic and viral abundance and production, and higher rates of organic matter decomposition. However, the differences between impact and control sediments were not consistent at all regions and habitats. Benthic bacterial diversity was always lower below the cages, where high viral-induced bacterial mortality rates were also observed. The δ- and γ-Proteobacteria dominated in both impacted and control sediments, but the relative importance of sulphate-reducing δ-Proteobacteria increased beneath the cages. We conclude that aquaculture can have a significant impact on benthic prokaryotes and viruses, by stimulating prokaryotic metabolism and viral infections, reducing bacterial diversity and altering assemblage composition. However, these impacts vary depending upon the sediment type and the habitat characteristics.

1. Introduction

Marine fish farming has increased exponentially worldwide in the last years (FAO, 2010) and is predicted to increase further over the next two decades (Duarte et al., 2009; Holmer, 2010). Aquaculture plants releasing large amounts of dissolved and particulate nutrients can have a significant impact on both the planktonic and benthic domains (Holmer and Kristensen, 1992; Pitta et al., 1999; Holmer et al., 2008; Sarà et al., 2011; Schneider et al., 2011). The extent of this impact is generally dependent upon the rates of organic matter accumulation on the sediment beneath the sea cages (Pusceddu et al., 2007; Holmer and Frederiksen, 2007), which causes a decrease in oxygen availability for the benthic assemblages and the increase of toxic products, such as sulphides and ammonium (Holmer and Kristensen, 1992; Holmer and Frederiksen, 2007). Previous studies demonstrated that these changes have a significant effect on the abundance, biodiversity and community structure of benthic organisms, including macrofauna (Apostolaki et al., 2007), macrophytes (Holmer et al., 2008), meiofauna (Mirto et al., 2000, 2010; Danovaro et al., 2004) and protists (Bongiorni et al., 2005).

Biodeposition from intensive aquaculture can influence also prokaryotes (Bacteria and Archaea) associated to farm sediments. Previous studies, indeed, have reported an increase in total prokaryotic abundance and biomass,

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prokaryotic carbon production and enzymatic activities in the sediments beneath the cages (Mirto et al., 2000; La Rosa et al., 2001; Vezzulli et al., 2002; La Rosa et al., 2004; Vezzulli et al., 2008; Caruso et al., 2003; Sakami et al., 2005). More recently, the potential effects of aquaculture on the diversity of prokaryotic assemblages have been investigated, with special attention on those involved in the sulphur cycling (Asami et al., 2005; Bissett et al., 2006, 2007; Kawahara et al., 2009).

To date no information is available on the effects of fish farming on benthic marine viruses. Benthic viruses play a crucial role in the functioning of benthic environments, by influencing biogeochemical cycling of key elements (particularly phosphorus, through the release of extracellular DNA; Dell'Anno and Danovaro, 2005) and ecosystem functioning (by controlling prokaryotic carbon production and converting biomass into dissolved organic matter; Weinbauer, 2004; Suttle, 2007; Corinaldesi et al., 2010). Their role in the oceans' functioning can also be amplified following the present global changes (Danovaro et al., 2011).

Such a lack of knowledge hampers a full comprehension of the response of the benthic assemblages to aquaculture impacts and, consequently, the prediction of the biodeposition effects on ecosystem functioning. To cope with this gap, we investigated four regions of the Mediterranean Sea characterized by the presence of intensive aquaculture plants. In each region, the impact of intensive fish farming on benthic viruses and prokaryotes and their interactions was assessed in two different habitats: (a) sediments within seagrass (*Posidonia oceanica*) meadows (hereafter defined as "vegetated sediments"); and (b) non vegetated sediments (hereafter defined as "unvegetated"). Pristine locations located far away from the plants, at the same depth and displaying the same habitat characteristics, were used as controls. Using a hierarchical sampling strategy, we tested the following (null) hypotheses: 1) the presence of fish farms does not influence benthic viral assemblages (in terms of abundance, production and prokaryotic mortality rates); 2) farm sediments have no impact on benthic prokaryotic assemblages (in terms of total abundance, activity, diversity and assemblage structure) and 3) the habitat type is influential for viral and prokaroytic assemblages and their interactions.

2. Materials and methods

2.1. Study areas and sampling activities

To test the abovementioned hypotheses, sediment sampling was performed in four regions located across a longitudinal gradient in the Mediterranean Sea (Fig. 1): Akrotiri Bay (Cyprus; 34°39′ N, 34°04′ E), Sounion Bay (southern Greece; 37°39′ N, 24°01′ E), Pachino Bay (Italy; 36°43′ N, 15°05′ E) and Gulf of Alicante (Spain; 38°24′ N, 0°24′ W). The four sampling regions were selected on the basis of the presence of the fish farms, which have been previously characterized in terms of their main environmental features (Table 1). Details about sampling activities and ecological and biogeochemical characteristics of the sediments at the study sites are summarized in Frederiksen et al. (2007), Holmer et al. (2008), Pusceddu et al. (2007) and Mirto et al. (2010). These studies highlighted that fish farming activities significantly affect the sedimentation rates of organic matter to the seafloor at all sites, and that biodeposition varied among the four sites due to differences in the hydrodynamic regime, water depth, food consumption and presence of wild fish (Frederiksen et al., 2007). Frederiksen et al. (2007) also highlighted that farming promote sulphide invasion in *P. oceanica* under the cages, and that the extent of seagrass decline vary significantly among sites according to the sediment biogeochemistry.

In each region, the effects of the fish farms were investigated in two different habitats: sediments covered by meadows of the *P. oceanica* seagrass, and soft-bottom unvegetated sediments. At each region, a preliminary survey was carried out to ascertain the presence of both the seagrass and the soft-bottom substrates, and to characterize the environmental settings of the areas in terms of mean depth and temperature, bottom currents, sediment type, porosity, chlorophyll-*a* and inorganic nutrient concentrations in the water column (Karakassis et al., 2005). Within each habitat, the impact of fish farming activities was quantified by contrasting the sediments below the fish cages (defined "impact" sediments) with control sites, located at least at 1000 m distance from the cages and upstream of the main water currents (Holmer et al., 2008). Controls were characterized by relatively pristine conditions and by environmental features comparable to those found beneath the fish farm cages (Mirto et al., 2010). Three independent replicates were collected randomly from the central area of each fish farm site (i.e., beneath the cages) and in each control site by scuba divers using sterile plexiglass corers (internal diameter 4.5 cm). Immediately after retrieval, the cores were transported into the laboratory for processing according to the specific protocols required for each microbiological variable. During transport, cores were kept at *in situ* temperature in the dark. Once in the laboratory, the top 1 cm of each corer was carefully extruded and processed as described below. For total prokaryotic abundance, sediment samples (ca. 1 cm^3) were transferred into sterile test tubes, fixed with 4 ml of pre-filtered (0.2 μm) and buffered 2% formalin and stored at 4 °C until analyses (within 1 week). For the determination of total viral abundance, in order to provide unbiased estimates of viral abundance induced by the use of preservatives, sediment aliquots (ca. 1 cm^3) from each core were transferred into sterile tubes, supplemented with 4.5 sterile virus-free seawater (prefiltered onto 0.02-μm membranes) and stored at 20 °C until analysis (within one week; Danovaro et al., 2008). For estimates of enzymatic activities and prokaryotic heterotrophic carbon production, aliquots of sediment were collected from the sediment core using sterile syringes and immediately analysed as described below. For molecular analyses of bacterial diversity, aliquots (ca. 10 cm^3) of sediments were put into polystyrene sterile 50-ml test tubes using sterile spatulas, immediately frozen and then stored at −20 °C until DNA extraction (within 1 week).

2.2. Total prokaryotic abundance

Total prokaryotic abundance was determined according to the SYBR Green Direct Count (SGDC) procedure described by Luna et al. (2002). Samples were sonicated three times (Branson Sonifier 2200, 60 W) for 1 min, properly diluted with 0.2 μm