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Rapid response of benthic deep-sea microbes (viruses and prokaryotes) to an intense dense shelf water cascading event in a submarine canyon of the NW Mediterranean Sea

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A B S T R A C T

A major dense shelf water cascading (DSWC) event occurred in 2005 downward the Cap de Creus Canyon (Gulf of Lion, NW Mediterranean Sea), which caused a significant change in environmental parameters and biological components. Here we describe the effects of this DSWC event on benthic microbes and on virus-prokaryote interactions, and we explore their implications on the functioning of the canyon's ecosystem. We collected sediment samples at increasing depths inside the canyon and in the adjacent deep continental margin over a period of five years, i.e. during and after the DSWC event, which led to the deposition of high amounts of fresh and labile organic matter that stimulated C production by benthic prokaryotes and increased their abundance and biomass. The enhanced prokaryotic metabolism, still evident 6 months after the DSWC event, was associated with high viral replication rates and prokaryotic mortality, which released 3.4–6.3 gC m^{-2} over such a 6 months period. Such values are up to 3-times higher than the yearly C-flux to the seafloor reported in this area in years without DSWC. We conclude that DSWC can significantly enhance benthic prokaryotic metabolism and C cycling through viral-induced prokaryotic mortality.

1. Introduction

Benthic deep-sea ecosystems represent more than 65% of the Earth's surface and provide goods and services that are vital for the entire biosphere, including C burial, nutrient cycling and biomass production (Barbier et al., 2014; Danovaro et al., 2014; Thurber et al., 2014). Biomass in this environment is dominated by prokaryotes, whose dynamics are dependent on a complex interplay of factors, including predatory pressure exerted by benthic fauna and virus-induced mortality, and changes of environmental conditions such as food availability, temperature or salinity (Danovaro et al., 2016a, 2016b, Danovaro et al., 2017a, 2017b). Increasing evidence shows that benthic ecosystems of continental margins are highly dynamic and also sensitive to environmental changes due to physical forcings and associated processes, like turbidity currents, open sea convection and dense shelf water cascading (DSWC) events (Liu et al., 2010; Fernandez-Arcaya et al., 2017). DSWC is an episodic phenomenon driven by atmospheric forcing, which results in the formation of dense coastal surface waters that generate buoyancy-driven currents overflowing the shelf edge and descending down the continental slope (Shapiro et al., 2003).

Deep-sea canyons are geomorphological features that can favour or even amplify the effects of DSWC (Canals et al., 2006; Allen and Durrieu de Madron, 2009). Indeed, submarine canyons can intercept and convey DSWC currents and the large amounts of materials they typically transport, thus acting as preferential conduits of mass and energy transfer from the coastal sea to the deep ocean interior (Canals et al., 2009; Xu, 2011; Fernandez-Arcaya et al., 2017). In this regard, it has been reported that the down-canyon channelling of DSWC currents can result in a significant increase of organic matter inputs down to bathyal depths (Canals et al., 2006; Pasqual et al., 2010), thus profoundly influencing the biodiversity and functioning of deep marine habitats (Durrieu de Madron et al., 2000; Bianchelli et al., 2008; Company et al., 2008; Pusceddu et al., 2013). As DSWC, also open ocean convection can potentially enhance biological activity in bathypelagic waters (Martini et al., 2013; Tamburini et al., 2013), due to deep-sea sediments resuspension (Durrieu de Madron et al., 2017). However, no information is available to date on the response to such events of benthic prokaryotic assemblages and the viruses infecting

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them.

In the Gulf of Lion, three major cascading events occurred in 1999, 2005 and 2006, with maximum bottom current velocities up to 1 m s^{-1} or even higher (Canals et al., 2006; Heussner et al., 2006; Palanques et al., 2012). During the DSWC event of 2005, one of the most intense events ever recorded in the study area, large amounts of fresh organic material originating from the continental shelf were transported down Lacaze-Duthiers Canyon and, mainly, Cap de Creus Canyon (CCC) to the deep margin and basin (Canals et al., 2006; Sanchez-Vidal et al., 2009). This DSWC event caused a decrease in the abundance and diversity of benthic deep-sea meiofaunal assemblages, likely due to the massive disturbance caused by the cascading flows (Pusceddu et al., 2013). The study presented here aimed at testing the hypothesis that intense DSWC events, such the one in early 2005, can also have a strong influence on virus-prokaryote interactions with cascade effects on the functioning of the microbial food webs and biogeochemical processes in benthic deep-sea ecosystems. To do so, we investigated changes in prokaryotic abundance, biomass and C production along with viral abundance and production and virus-induced prokaryotic mortality inside CCC, NW Mediterranean Sea, over a period of five years, during and after the major 2005 DSWC event.

2. Materials and methods

2.1. Study area and sampling sites

The study area is located in the Gulf of Lion, which includes one of the most intricate networks of submarine canyons of the Mediterranean Sea (Canals et al., 2006, 2013). Some canyons extend for greater than 100 km, cutting the entire continental slope and reaching depths in excess of 2000 m (Amblas et al., 2006; Canals et al., 2009). Among these, the Cap de Creus Canyon (CCC) incises the westernmost Gulf of Lion continental shelf and slope before opening into the larger Sète Canyon (Lastras et al., 2007). In late winter–early spring 2005, a particularly intense DSWC occurred with dense waters overflowing the shelf edge and flowing down the continental slope and CCC down to the deep margin and basin at depths larger than 2000 m, causing a sudden drop in deep-sea temperature (from approximately 13 °C down to 10 °C at 750 m depth; Canals et al., 2006). In the CCC, such event was associated with an increase in bottom current speed (with peaks in excess of 1 m s^{-1}), in water density and in sediment transport, resulting in an estimated overall organic C export of 0.6 million tons in less than two months (Canals et al., 2006). Sediment sampling was carried out along the axis of CCC and in the adjacent deep margin during five oceanographic cruises carried out during (April 2005) and after (October 2005, August 2006, April 2008 and April 2009) the late-winter/early-spring 2005 DSWC event (Fig. 1). Sediment samples were collected with a multicorer at 1000 m and 1800 m depth. Additional samples were obtained at depths larger than 2100 m in April 2005, October 2005, August 2006 and April 2009. At each investigated site, the top 1 cm of three independent sediment cores was subsampled and analyzed for phytopigment concentrations (as a proxy of the most fresh and labile organic matter settling to the seafloor), prokaryotic abundance, biomass and heterotrophic C production, as well as for viral abundance, viral production and virus-induced prokaryotic mortality.

2.2. Phytopigment concentration

Chlorophyll-a and phaeopigments were analyzed fluorometrically according to standard protocols (Danovaro, 2010). Pigments were extracted from triplicate sediment samples using 90% (vol/vol) acetone (12 h in the dark at 4 °C). After centrifugation, the supernatant was used to determine chlorophyll-a concentrations and acidified with 0.1 N HCl in order to determine phaeopigment concentrations. Total phytopigment concentrations were obtained from the sum of chlorophyll-a and phaeopigment concentrations (Danovaro, 2010).

2.3. Prokaryotic abundance and biomass

The total prokaryotic abundance was determined by epifluorescence microscopy according to standard procedures (Danovaro, 2010). Briefly, samples were sonicated three times with a Branson Sonifier 2200, 60 W, for 1 min, properly diluted with sterile and 0.2 μm pre-filtered seawater and then 3 ml of each sample were filtered onto 0.2 μm pore-size Al_2O_3 Anodisc filters (Whatman). Filters were then stained with SYBR Green I (Molecular Probes) by adding, on each filter, 20 μl of the stock solution (previously diluted 1:20 with filtered [0.2-μm-pore-size] Milli-Q water), washed twice with 3 ml of sterilized Milli-Q water and mounted onto microscope slides. Filters were analyzed using epifluorescence microscopy (Zeiss Axioskop 2MOT, magnification ×1000). For each filter, at least 20 microscope fields were observed and at least 400 cells were counted. Data were normalized to sediment dry weight after desiccation (48 h at 60 °C). For the determination of the prokaryotic biomass, the cell biovolume obtained from prokaryotic size following inter-calibration with scanning electron microscopy-based size determinations was converted into C content assuming 310 fg C μm^{-3} (Fry, 1990) in line with previous studies (Danovaro, 2010 and references therein; Rastelli et al., 2016).

2.4. Prokaryotic heterotrophic C production

For the determination of prokaryotic heterotrophic C production, sediment sub-samples were incubated with ^{3}H-leucine (specific activity, 68 Ci $mmol^{-1}$; final concentration, 0.2 μM), previously diluted in virus-free seawater collected from the water–sediment interface, for 1 h in the dark at in-situ temperature. Time-course experiments over 6 h and concentration-dependent incorporation experiments (from 0.05 μM to 5.0 μM leucine) were also carried out to define the linearity of the ^{3}H-leucine incorporation and to estimate the leucine saturation level, respectively. After incubation, samples were supplemented with ethanol (80%), centrifuged, washed again two times with ethanol (80%), and the sediment was finally re-suspended in ethanol (80%) and filtered onto polycarbonate filters (0.2 μm pore size; vacuum < 100 mm Hg). Subsequently, each filter was washed four times with 2 ml of 5% TCA, then transferred into a Pyrex tube containing 2 ml of NaOH (2 M) and incubated for 2 h at 100 °C. After centrifugation at 800*g*, 1 ml of supernatant fluid was transferred to vials containing an appropriate scintillation liquid. Sediment blanks were made by adding ethanol (80%) immediately before the ^{3}H-leucine addition and processed as described above. The incorporated radioactivity in the sediment samples was measured with a liquid scintillation counter Packard Tri-Carb 2100 (Luna et al., 2013; Rastelli et al., 2015). The prokaryotic heterotrophic C production was calculated as follows:

$$\textit{Prokaryotic heterotrophic C production} = LI \times 131.2 \times (\%Leu)^{-1} \times (C/protein) \times ID$$

where *LI* is the leucine incorporation rate (mol g^{-1} h^{-1}), *131.2* is the molecular weight of leucine, *%Leu* is the fraction of leucine in a protein (0.073), *C/protein* is the ratio of cellular C to protein (0.86), and *ID* is the isotope dilution, assumed to be 2 (Simon and Azam, 1989). The isotope dilution value we used has been largely applied to determine prokaryotic heterotrophic C production in deep-sea sediments collected worldwide (Danovaro et al., 2008), thus allowing a proper comparison.

2.5. Viral abundance, production and virus-induced prokaryotic mortality

Viral abundance was determined after the detachment of viruses from the sediment using pyrophosphate (final concentration, 5 mM) and ultrasound treatment (Danovaro, 2010). Samples were diluted 100–500-fold with sterile and virus-free water (filtered through 0.02-μm-pore-size filters), treated with DNases (to remove extracellular DNA) and filtered onto 0.02 μm pore size filters (Anodisc Al_2O_3, 25 mm