Amended version of the manuscript PROOCE_2018_94

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

Eugenio Rastelli[1*], Cinzia Corinaldesi[2*], Miquel Canals[3], Roberto Danovaro[1,4], Antonio Dell'Anno[4]

[1]*Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy*

[2]*Department of Sciences and Engineering of Materials, Environment and Urbanistics, Polytechnic University of Marche, 60131 Ancona, Italy*

[3]*CRG Marine Geosciences, Department of Earth and Ocean Dynamics, Faculty of Earth Sciences, University of Barcelona, E-08028 Barcelona, Spain*

[4]*Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona Italy*

[*]**Corresponding authors**:
Eugenio Rastelli
Department of Integrative Marine Ecology (EMI)
Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy.
Tel: +39 081 5833221
e-mail: eugenio.rastelli@szn.it

Cinzia Corinaldesi
Department of Sciences and Engineering of Materials, Environment and Urbanistics (SIMAU)
Polytechnic University of Marche, Via Brecce Bianche, 60131, Ancona, Italy.
Tel: +39 071 2204294
e-mail: c.corinaldesi@univpm.it

Abstract

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.

Keywords: virus-host interaction; prokaryotic metabolism; deep-sea sediments; canyon ecosystem functioning

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; Danovaro et al.,2016b, Danovaro et al., 2017a; Danovaro et al.,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 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 > 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 2000m, 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 (Figure 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, 60W, for 1 minute, 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 × 1,000). 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 hours 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 (2M) 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:

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

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 diameter). The filters were stained using SYBR Green I (10000× in anhydrous dimethyl sulfoxide, Molecular Probes-Invitrogen), incubated in the dark for 20 min and mounted on glass slides with a drop of 50% phosphate buffer (6.7 mmol L^{-1}; pH 7.8) and 50% glycerol containing 0.5% ascorbic acid. Viral counts were performed under epifluorescence microscopy, by examining at least 10 fields per slide and counting at least 400 viral particles per filter. Viral production was determined by time-course experiments of sediment samples previously diluted with virus-free seawater (0.02 µm pre-filtered), collected at the sediment-water interface of each benthic site (Dell'Anno et al., 2009; Rastelli et al., 2016). A standard dilution of sediment samples with virus-free seawater was used (sediment to virus-free seawater 1:10 vol:vol). Replicate samples (n=3) for viral counts were collected immediately after dilution of the sediments and after 3, 6 and 12 h of incubation in the dark at in-situ temperature. Subsamples were then analyzed as reported for the determination of viral abundance. In all of the samples, the viral production was determined from linear regression analyses of the increase of viral abundances versus time. Prokaryotic burst size (BS, i.e, the number of viruses released by each cell lysed due to viral infection) was estimated from time-course experiments of viral production following Mei and Danovaro (2004), and using the equation: $BS = VP / Pkilled$ where VP is the number of viruses produced g^{-1} h^{-1}, determined as described above for the assessment of viral production rates by epifluorescence microscopy, and *Pkilled* is the number of prokaryotic cells killed g^{-1} h^{-1}. *Pkilled* was estimated as follows:

$$P_{killed} = (P_{start} + P_{prod}) - (P_{end})$$

where P_{start} is the prokaryotic abundance at start of incubations as determined by epifluorescence microscopy (see methods above); P_{prod} is the number of prokaryotic cells produced in the interval of incubation calculated as prokaryotic C production (determined by the radiotracer incubation

experiments as described above) divided by prokaryotic biomass per cell (see methods above for details on biomass estimates); and P_{end} is the number of prokaryotes actually counted after the incubation interval by epifluorescence microscopy (Mei and Danovaro, 2004; Danovaro et al., 2008). The amount of C released by viral lysis during the period from April 2005 to October 2005 (i.e., over the 6 months following the major cascading event) was estimated as the C of the overall P_{killed} over that period. For comparison with POC fluxes at the seafloor (Gogou et al., 2014), the amount of C released by viral lysis has been expressed per square meter by assuming a sediment density of 1.8 and 50% water content (Dell'Anno and Danovaro 2005).

The virus-induced prokaryotic mortality was calculated following Rastelli et al., 2016 as:

$$(P_{killed} / P_{prod}) \times 100$$

which is dividing the number of cells killed by viruses g^{-1} h^{-1} by the total number of prokaryotes produced g^{-1} h^{-1}, and multiplying per 100 to express the value as percentage.

2.6 Statistical analyses. To test the responses of prokaryotes and viruses to the DSWC event, we used both uni- and multivariate permutational non-parametric analyses of variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001). We determined the effects of the cascading on each variable separately. The multivariate design included two orthogonal factors: sampling time (5 fixed levels) and water depth (2 fixed levels: 1000 m and 1800m depth). Pairwise comparison tests were also carried out to discriminate the effects of DSWC at each depth. Since the information for the deep margin did not include data from April 2008, to avoid unbalanced designs a separate one-way test (with sampling time as the unique source of variation with 4 fixed levels, April 2005, October 2005, August 2006 and April 2009) was carried out to ascertain the effects of cascading at >2100 m depth in the deep margin. All statistical tests were carried out using the PRIMER6+ software.

3. Results and discussion

There is evidence that the amount and distribution of organic matter in continental margin sediments are highly variable. They depend not only on particle settling from overlying waters, but also on a wide array of processes, including down-canyon/slope advective transport; stirring, re-suspension and re-sedimentation of particles; and episodic events such as gravity-driven sediment flows and DSWC (Canals et al., 2006; Heussner et al., 2006; de Stigter et al., 2007; Dell'Anno et al., 2013). In this study, we report significant spatial and temporal changes of photosynthetically-produced organic matter in the uppermost layer of seafloor sediments from CCC and the adjacent deep margin (Figure 2). We found a significant increase of phytopigment concentrations in deep

basin the sediments and depletion at 1000 m depth associated with the 2005 DSWC event. Such a pattern reinforces previous findings obtained from the same area showing an enhanced export of fresh and labile organic materials originating from the seasonal spring phytoplankton bloom in 2005 towards the deep margin and basin (Canals et al., 2006; Pusceddu et al., 2013). These findings suggest that submarine physiography and high-energy hydrodynamic processes exert an important control on the pelagic-benthic coupling in continental margin systems.

In food-limited ecosystems, such as the deep sea, the amount of labile organic materials deposited on the seafloor profoundly influences the abundance, biomass and metabolism of benthic prokaryotes (Dell'Anno and Danovaro 2005; Jorgensen and Boetius 2007; Danovaro et al., 2014). Our results show that, during the 2005 DSWC event, benthic prokaryotic abundance, biomass and heterotrophic C production were significantly higher in surface sediments at >2100 m water depth compared with values at shallower water depths and such an effect was still evident 6 months after the end of the event (i.e. in October 2005; Figure 3A-C). An enhanced prokaryotic standing stock and metabolism were also observed in April 2005 compared to the other periods at 1800 m of water depth, despite relatively low food availability on the seafloor at that time.

The positive effect of DSWC on microbial assemblages was opposite to what had been previously reported in the same study sites for the abundance and diversity of meiofauna, which dropped significantly in April 2005 at all depths (Pusceddu et al., 2013). These results suggest that intense DSWC events, increasing food availability (bottom-up effect) and decreasing predatory pressure exerted by benthic metazoans (top-down effect), amplify the responses of the benthic microbial food webs, even on a relatively long time scale (i.e. months).

In benthic deep-sea ecosystems, viral infections exert a major control on prokaryotic dynamics (Danovaro et al., 2008; Danovaro et al., 2016a). In this study, viral abundances (Figure 4A) displayed the same spatial and temporal patterns than prokaryotic abundances, indicating, for the first time, a tight virus-host interaction in dynamic and physiographically complex benthic deep-sea ecosystems, such as the submarine canyon and adjacent deep continental margin investigated here. The values of viral abundances in the sediments of the deep margin during the major 2005 DSWC event were among the highest reported so far in deep seabed surface sediments worldwide (Danovaro et al., 2008; Danovaro et al., 2015; Danovaro et al., 2016a; Rastelli et al., 2016) and comparable to those found in highly productive coastal areas (Parikka et al., 2016).

Viral abundances in the sediments depend on the balance between viral production and decay rates (Corinaldesi et al., 2010; Dell'Anno et al., 2015). Here, we provide evidence of a major increase of benthic viral production associated with the 2005 DSWC event both at 1800 m depth and in the

deep basin (Figure 4B). Such an increase indicates the occurrence of fast viral replication rates sustained by the enhanced metabolism of the benthic prokaryotic hosts (Figure 3C). We also found that during the DSWC event, virus-induced prokaryotic mortality was very high and the effects were evident also 6 months after the end of the event (i.e., in October 2005; Figure 4C). We estimated that prokaryotic mortality induced by viral lysis in the deep Gulf of Lion caused the release of 3.4-6.3 gC m^{-2} over only 6 months (from April 2005 to October 2005). This C amount released by prokaryotic mortality is up to three times higher than the yearly C-flux to the seafloor reported for this area in years without cascading (Gogou et al., 2014). This finding suggests that this process should be included in the C budget at regional scale.

The high virus-induced prokaryotic mortality can have additional ecological consequences: on one hand, the higher viral infections, by diverting a larger fraction of prokaryotic biomass into organic detritus, can represent an additional important trophic resource able to sustain the growth of uninfected microbes, thus accelerating C cycling (Danovaro et al., 2008, Danovaro et al., 2016a; Rastelli et al., 2016, Rastelli et al., 2017). On the other hand, it can significantly reduce the transfer of energy and material to the higher trophic levels.

Overall, our findings indicate that DSWC events, conveying large amounts of trophic resources mainly along submarine canyons to the deep sea and reducing metazoan components, can significantly stimulate benthic microbial assemblages and viral infection processes, thus strongly influencing C and nutrient cycling in the deep seafloor. The Mediterranean Sea is a semi-enclosed, so-called “miniature ocean”, which is particularly sensitive to the effects of climate change (Lejeusne et al., 2010; Philippart et al., 2011). Here, as well as in other mid- and high-latitude regions of the world, the intensity and frequency of extreme weather events are expected to increase due to climate change (Somot et al., 2006; Coma et al., 2009; Thomsen et al., 2012). Therefore, an intensification of the frequency and intensity of DSWC events can be predicted along with a possible extension of deep-sea regions affected by this phenomenon (Canals et al., 2006; Pusceddu et al., 2013). The results of the present study provide additional insights into the analysis of the ecological effects of DSWC events on the functioning of benthic deep-sea ecosystems. Although our study was conducted on a single canyon and adjacent deep margin and, therefore, it does not allow inferring the response of all deep-sea ecosystems impacted by DSWC, the effects of the alteration of benthic microbial processes on C storage and cycling in deep-sea benthic ecosystems could be highly relevant and deserve further investigations elsewhere.

Acknowledgements. We thank Marianna Mea for the support during sampling activity and Andrea Fioretti for the contribution to part of the laboratory analyses. This research was supported by the Collaborative Project HERMIONE, EC contract no. 226354, under the European Commission's 7th Framework Programme. MC acknowledges support to CRG Marine Geosciences by the Autonomous Government of Catalonia under its grant 2017 SGR 315.

Author Contributions: E.R., C.C., R.D. and A.D. conceived the study. E.R., C.C. and A.D. contributed to data elaboration and interpretation. E.R., C.C. and A.D. wrote the first draft of the manuscript. All authors contributed to results discussion and finalization of the manuscript.

Conflict of interest: All the other authors declare no competing financial interests.

References

Allen, S.E., Durrieu de Madron, X., 2009. A review of the role of submarine canyons in deep-ocean exchange with the shelf. Ocean Science, 5 (4), 607-620. DOI: 10.5194/os-5-607-2009.

Amblas, D., Canals, M., Urgeles, R., Lastras, G., Liquete, C., Hughes-Clarke, J.E., Casamor, J.L., Calafat, A.M., 2006. Morphogenetic mesoscale analysis of the northeastern Iberian margin, NW Mediterranean Basin. Marine Geology, 234 (1-4), 3-20. DOI: 10.1016/j.margeo.2006.09.009.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecology, 26, 32–46. DOI: 10.1111/j.1442-9993.2001.01070.pp.x

Barbier, E.B., Moreno-Mateos, D., Rogers, A.D., Aronson, J., Pendleton, L., Danovaro, R., Henry, L.-A., Morato, T., Ardron, J., Van Dover, C.L., 2014. Protect the deep sea. Nature,505 (7484), 475-477. DOI: 10.1038/505475a.

Bianchelli, S., Gambi, C., Pusceddu, A., Danovaro, R., 2008. Trophic conditions and meiofaunal assemblages in the Bari Canyon and the adjacent open slope (Adriatic Sea). Chemistry and Ecology, 24 (S1), 101-109. DOI: 10.1080/02757540801963386.

Canals, M., Company, J.B., Martin, D., Sanchez-Vidal, A., Ramirez-Llodra, E., 2013. Integrated study of Mediterranean deep canyons: novel results and future challenges. Progress in Oceanography, 118, 1-27. DOI: 10.1016/j.pocean.2013.09.004.

Canals, M., Danovaro, R., Heussner, S., Lykousis, V., Puig, P., Trincardi, F., Calafat, A.M., Durrieu de Madron, X., Palanques, A., Sanchez-Vidal, A., 2009. Cascades in Mediterranean submarine grand canyons. Oceanography, 22 (1), 26-43. DOI: http://www.jstor.org/stable/24860920.

Canals, M., Puig, P., Durrieu de Madron, X., Heussner, S., Palanques, A., Fabres, J., 2006. Flushing submarine canyons. Nature, 444 (7117), 354. DOI: 10.1038/nature05271.Coma, R., Ribes, M., Serrano, E., Jimenez, E., Salat, J., Pascual, J., 2009. Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proceedings of the National Academy of Sciences,106 (15), 6176-6181. DOI: 10.1073/pnas.0805801106.

Company, J.B., Puig, P., Sardà, F., Palanques, A., Latasa, M., Scharek, R., 2008. Climate Influence on Deep Sea Populations. PLoS ONE, 3, e1431. DOI: 10.1371/journal.pone.0001431.

Corinaldesi, C., Dell'Anno, A., Magagnini, M., Danovaro, R., 2010. Viral decay and viral production rates in continental shelf and deep-sea sediments of the Mediterranean Sea. FEMS Microbiology Ecology, 72 (2), 208-218. DOI: 10.1111/j.1574-6941.2010.00840.x.

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C., Weinbauer, M., 2008. Major viral impact on the functioning of benthic deep-sea ecosystems. Nature, 454 (7208), 1084. DOI: doi:10.1038/nature07268.

Danovaro, R., 2010. Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC Press, Taylor & Francis Group, Boca Raton, 428 pp..

Danovaro, R., Snelgrove, P.V., Tyler, P., 2014. Challenging the paradigms of deep-sea ecology. Trends in Ecology & Evolution, 29 (8), 465-475. DOI: 10.1016/j.tree.2014.06.002.

Danovaro, R., Corinaldesi, C., Rastelli, E., & Anno, A. D. 2015. Towards a better quantitative assessment of the relevance of deep-sea viruses, Bacteria and Archaea in the functioning of the ocean seafloor. Aquatic Microbial Ecology, 75(1), 81-90.

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Rastelli, E., Cavicchioli, R., Krupovic, M., Noble, R.T., Nunoura, T., Prangishvili, D., 2016a. Virus-mediated archaeal hecatomb in the deep seafloor. Science Advances, 2 (10), e1600492. DOI: 10.1126/sciadv.1600492.

Danovaro, R., Molari, M., Corinaldesi, C., Dell'Anno, A., 2016b. Macroecological drivers of archaea and bacteria in benthic deep-sea ecosystems. Science Advances, 2 (4), e1500961. DOI: 10.1126/sciadv.1500961.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., & Rastelli, E. 2017a. Potential impact of global climate change on benthic deep-sea microbes. FEMS microbiology letters, 364(23), fnx214. DOI:10.1093/femsle/fnx214.

Danovaro, R., Rastelli, E., Corinaldesi, C., Tangherlini, M., & Dell'Anno, A. 2017b. Marine archaea and archaeal viruses under global change. F1000Research, 6, 1241-1241. DOI:10.12688/f1000research.11404.1.

Dell'Anno, A., Corinaldesi, C., Magagnini, M., Danovaro, R., 2009. Determination of viral production in aquatic sediments using the dilution-based approach. Nature Protocols, 4 (7), 1013-1022. DOI: 10.1038/nprot.2009.82.

Dell'Anno, A., Danovaro, R., 2005. Extracellular DNA plays a key role in deep-sea ecosystem functioning. Science, 309 (5744), 2179-2179. DOI: 10.1126/science.1117475.

Dell'Anno, A., Pusceddu, A., Corinaldesi, C., Canals, M., Heussner, S., Thomsen, L., Danovaro, R., 2013. Trophic state of benthic deep-sea ecosystems from two different continental margins off Iberia. Biogeosciences, 10 (5), 2945-2957. DOI: 10.5194/bg-10-2945-2013.

Durrieu de Madron, X., Abassi, A., Heussner, S., Monaco, A., Aloisi, J.C., Radakovitch, O., Giresse, P., Buscail, R., Kerherve, P., 2000. Particulate matter and organic carbon budgets for the Gulf of Lions (NW Mediterranean). Oceanologica Acta, 23 (6), 717-730. DOI: 10.1016/S0399-1784(00)00119-5.

Durrieu de Madron X, Ramondenc S, Berline L, Houpert L, Bosse A, Martini S, Guidi L, Conan P, Curtil C, Delsaut N, Kunesch S, Ghiglione JF, Marsaleix P, Pujo-Pay M, Séverin T, Testor P, Tamburini C and the ANTARES collaboration, 2017. Deep sediment resuspension and thick nepheloid layer generation by open-ocean convection. Journal of Geophysical Research: Oceans 122, 2291–2318

Fernandez-Arcaya, U., Ramirez-Llodra, E., Aguzzi, J., Allcock, A.L., Davies, J.S., Dissanayake, A., Harris, P., Howell, K., Huvenne, V.A.I., Macmillan-Lawler, M., Martin, J., Menot, L., Nizinski, M., Puig, P., Rowden, A.A., Sanchez, F., Van den Beld, I.M.J., 2017. Ecological role of submarine canyons and need for canyon conservation: a review. Frontiers in Marine Science,4, 5. DOI: 10.3389/fmars.2017.00005.

Fry, J.C., 1990. Direct methods and biomass estimation. In Grigorova, R. and Norris, J.R. (Eds.), Methods in Microbiology. Academy Press Limited, London, vol. 22, pp. 41–85.

Gogou, A., Sanchez-Vidal, A., Durrieu de Madron, X., Stavrakakis, S., Calafat, A.M., Stabholz, M., Psarra, S., Canals, M., Heussner, S., Stavrakaki, I., Papathanassiou, E., 2014. Carbon flux to the deep in three open sites of the Southern European Seas (SES). Journal of Marine Systems, 129, 224-233. DOI: 10.1016/j.jmarsys.2013.05.013.

Heussner, S., Durrieu de Madron, X., Calafat, A., Canals, M., Carbonne, J., Delsaut, N., Saragoni, G., 2006. Spatial and temporal variability of downward particle fluxes on a continental slope: Lessons from an 8-yr experiment in the Gulf of Lions (NW Mediterranean). Marine Geology, 234 (1-4), 63-92. DOI: 10.1016/j.margeo.2006.09.003.

Jorgensen, B.B., Boetius, A., 2007. Feast and famine--microbial life in the deep-sea bed. Nature Reviews Microbiology, 5 (10), 770-782. DOI: 10.1038/nrmicro1745.

Lastras, G., Canals, M., Urgeles, R., Amblas, D., Ivanov, M., Droz, L., Dennielou, B., Fabres, J., Schoolmeester, T., Akhmetzhanov, A., Orange, D., Garcia-Garcia, A., 2007. A walk down the

Cap de Creus canyon, Northwestern Mediterranean Sea: Recent processes inferred from morphology and sediment bedforms. Marine Geology, 246 (2-4), 176-192. DOI: 10.1016/j.margeo.2007.09.002.

Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C.F., Perez, T., 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends in Ecology & Evolution, 25 (4), 250-260. DOI: 10.1016/j.tree.2009.10.009.

Liu, K-K., Atkinson, L., Quinones, R., Talaue-McManus, L., 2010. Carbon and nutrient fluxes in continental margins: a global synthesis. Springer-Verlag, Berlin Heidelberg.

Luna, G. M., Corinaldesi, C., Rastelli, E., & Danovaro, R. 2013. Patterns and drivers of bacterial α- and β-diversity across vertical profiles from surface to subsurface sediments. Environmental microbiology reports, 5(5), 731-739. DOI:10.1111/1758-2229.12075.

Martini, S., Nerini, D., Tamburini, C., 2013. Relation between deep bioluminescence and oceanographic variables: A statistical analysis using time-frequency decompositions. Prog Oceanogr 127, 117–128.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis, Ecology, 82, 290–297. DOI: 10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2.

Mei, M.L. Danovaro, R., 2004. Virus production and life strategies in aquatic sediments. Limnology and Oceanography, 49 (2), 459-470. DOI: 10.4319/lo.2004.49.2.0459.

Palanques, A., Puig, P., Durrieu de Madron, X., Sanchez-Vidal, A., Pasqual, C., Martín, J., Calafat, A.M., Heusner, S., Canals, M., 2012. Sediment transport to the deep canyons and open-slope of the western Gulf of Lions during the 2006 intense cascading and open-sea convection period. Progress in Oceanography, 106, 1-15. DOI: 10.1016/j.pocean.2012.05.002.

Parikka, K.J., Le Romancer, M., Wauters, N., Jacquet, S., 2017. Deciphering the virus to prokaryote ratio (VPR): insights into virus–host relationships in a variety of ecosystems. Biological Reviews, 92 (2), 1081-1100. DOI: 10.1111/brv.12271

Pasqual, C., Sanchez-Vidal, A., Zuñiga, D., Calafat, A., Canals, M., Durrieu de Madron, X., Puig, P., Heussner, S., Palanques, A., Delsaut, N., 2010. Flux and composition of settling particles across the continental margin of the Gulf of Lion: the role of dense shelf water cascading. Biogeosciences, 7, 217-231. DOI: 10.5194/bg-7-217-2010.

Philippart, C.J., Anadon, R., Danovaro, R., Dippner, J.W., Drinkwater, K.F., Hawkins, S.J., Oguz, T., O'Sullivan, G., Reid, P.C., 2011. Impacts of climate change on European marine ecosystems: observations, expectations and indicators. Journal of Experimental Marine Biology and Ecology, 400 (1-2), 52-69. DOI: 10.1016/j.jembe.2011.02.023.

Pusceddu, A., Mea, M., Canals, M., Heussner, S., Durrieu de Madron, X., Sanchez-Vidal, A., Bianchelli, S., Corinaldesi, C., Dell'Anno, A., Thomsen, L., Danovaro, R., 2013. Major consequences of an intense dense shelf water cascading event on deep-sea benthic trophic conditions and meiofaunal biodiversity. Biogeosciences, 10 (4), 2659-2670. DOI: 10.5194/bg-10-2659-2013.

Rastelli, E., Corinaldesi, C., Dell'Anno, A., Amaro, T., Queirós, A. M., Widdicombe, S., & Danovaro, R. 2015. Impact of CO_2 leakage from sub-seabed carbon dioxide capture and storage (CCS) reservoirs on benthic virus–prokaryote interactions and functions. Frontiers in microbiology, 6, 935. DOI:10.3389/fmicb.2015.00935.

Rastelli, E., Dell'Anno, A., Corinaldesi, C., Middelboe, M., Noble, R.T., Danovaro, R., 2016. Quantification of viral and prokaryotic production rates in benthic ecosystems: A methods comparison. Frontiers in Microbiology, 7: 1501. DOI: 10.3389/fmicb.2016.01501.

Rastelli, E., Corinaldesi, C., Dell'Anno, A., Tangherlini, M., Martorelli, E., Ingrassia, M., Chiocci, F.L, Lo Martire, M. & Danovaro, R. 2017. High potential for temperate viruses to drive carbon cycling in chemoautotrophy dominated shallow-water hydrothermal vents. Environmental microbiology, 19(11), 4432-4446. DOI:10.1111/1462-2920.13890.

Sanchez-Vidal, A., Pasqual, C., Kerherve, P., Heussner, S., Calafat, A., Palanques, A., Durrieu de Madron, X., Canals, M., Puig, P., 2009. Across margin export of organic matter by cascading events traced by stable isotopes, northwestern Mediterranean Sea. Limnology and Oceanography, 54 (5), 1488-1500. DOI: 10.4319/lo.2009.54.5.1488.

Shapiro, G.I., Huthnance, J.M., Ivanov, V.V., 2003. Dense water cascading off the continental shelf. Journal of Geophysical Research: Oceans, 108: 3390. DOI: 10.1029/2002JC001610.

Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series, 51: 201-213. DOI: http://www.jstor.org/stable/24833670.

Somot, S., Sevault, F., Déqué, M., 2006. Transient climate change scenario simulation of the Mediterranean Sea for the twenty-first century using a high-resolution ocean circulation model. Climate Dynamics, 27 (7-8), 851-879. DOI: 10.1007/s00382-006-0167-z.

de Stigter, H.C., Boer, W., de Jesus Mendes, P.A., Jesus, C.C., Thomsen, L., van den Bergh, G.D., van Weering, T.C., 2007. Recent sediment transport and deposition in the Nazaré Canyon, Portuguese continental margin. Marine Geology, 246 (2-4), 144-164. DOI: 10.1016/j.margeo.2007.04.011.

Tamburini C, Canals M, Durieu de Madron X, Houpert L, Lefèvre D, Martini S, D'Ortenzio F, Robert A, Testor P and the ANTARES collaboration, 2013. Deep-sea bioluminescence blooms after dense water formation at the ocean surface. PLoS One 8, e67523.

Thomsen, L., Barnes, C., Best, M., Chapman, R., Pirenne, B., Thomson, R., Vogt, J., 2012. Ocean circulation promotes methane release from gas hydrate outcrops at the NEPTUNE Canada Barkley Canyon node. Geophysical Research Letters, 39, L16605. DOI: 10.1029/2012GL052462.

Thurber, A.R., Sweetman, A.K., Narayanaswamy, B.E., Jones, D.O.B., Ingels, J. Hansman, R.L., 2014. Ecosystem function and services provided by the deep sea. Biogeosciences, 11 (14), 3941-3963. DOI: 10.5194/bg-11-3941-2014.

Xu, J.P., 2011. Measuring currents in submarine canyons: Technological and scientific progress in the past 30 years. Geosphere, 7 (4), 868-876. DOI: 10.1130/GES00640.1.

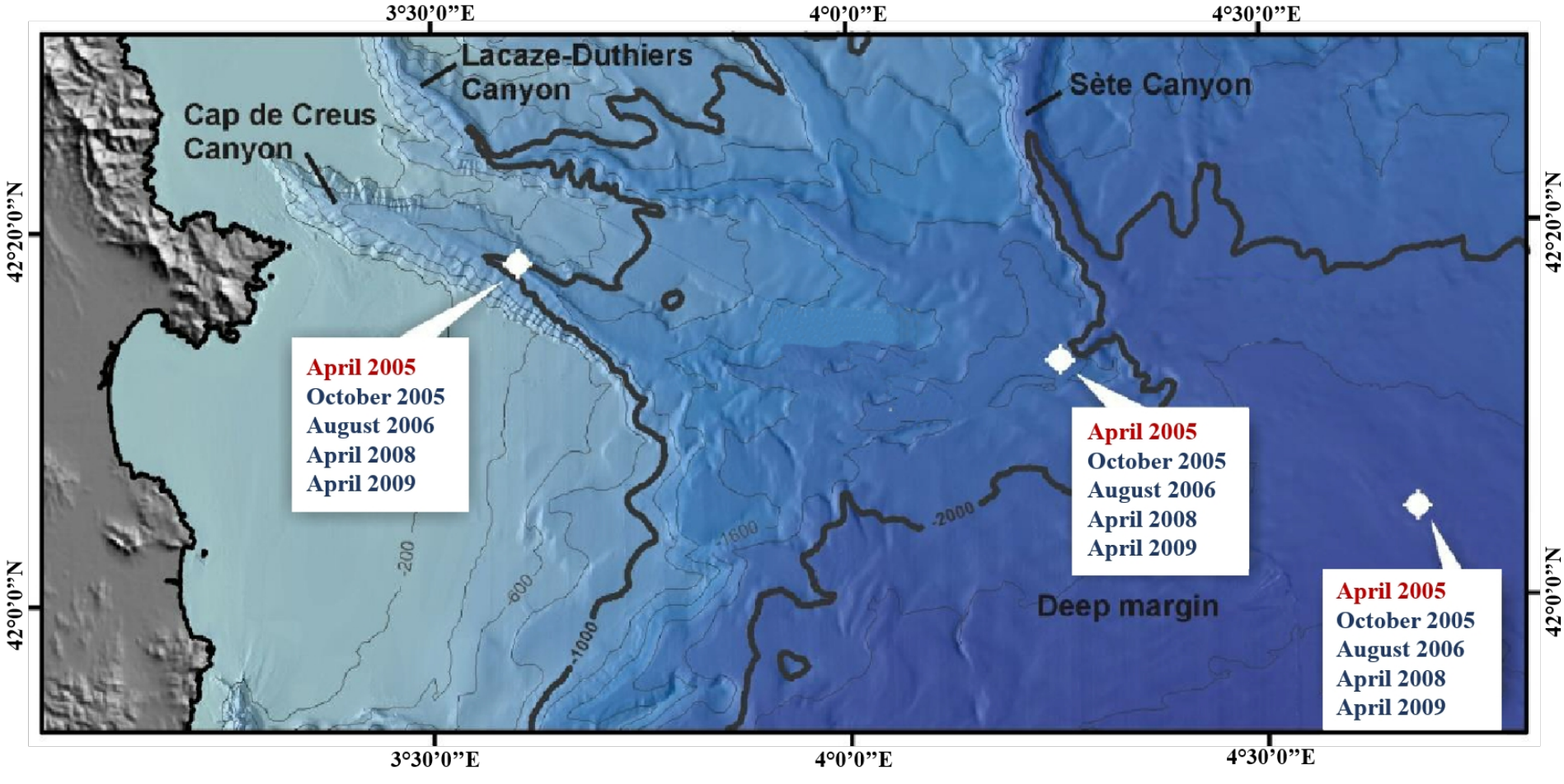
Figure captions

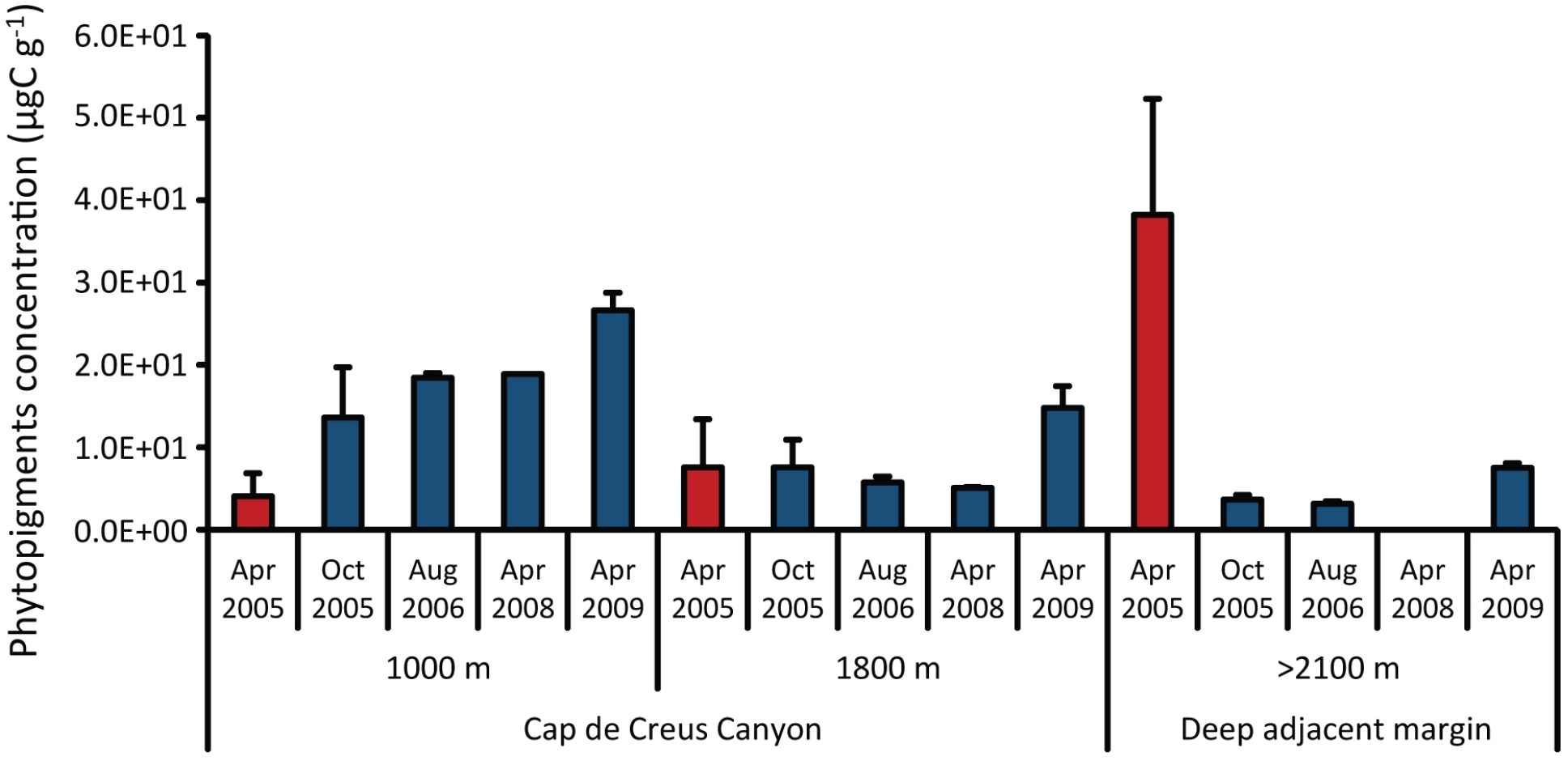
Figure 1. Location of sampling sites in the Cap de Creus Canyon and the deep adjacent margin. Diamonds represent the stations investigated during different sampling periods. April 2005 is highlighted in red to indicate intense DSWC occurrence (i.e. in late winter-early spring 2005). Reported are also sampling times for each investigated site. Contours in meters.

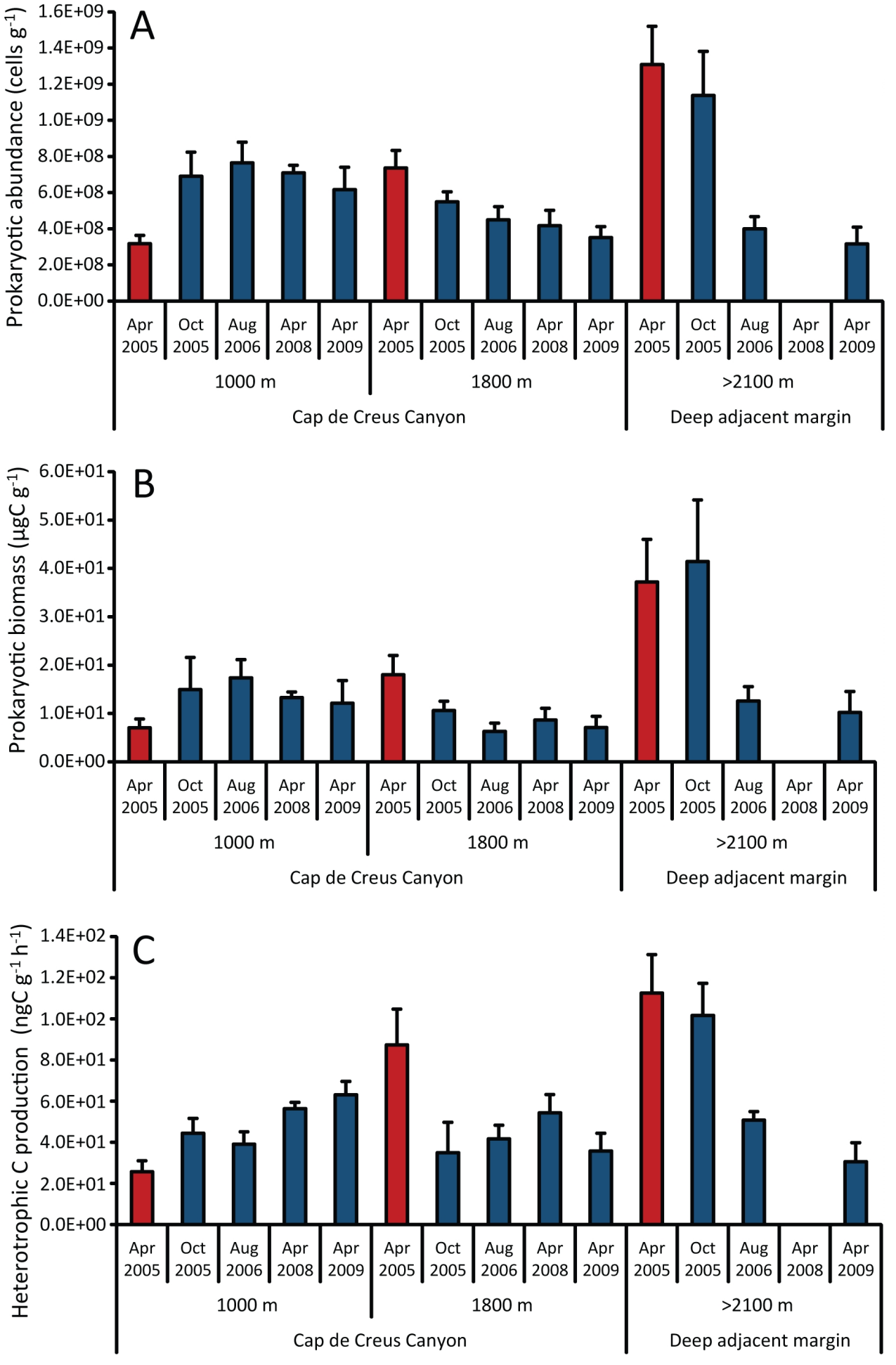
Figure 2. Spatial and temporal changes of phytopigment concentrations in surface sediments of the Cap de Creus Canyon and the deep adjacent margin. Reported are mean and standard deviations. Red columns indicate intense DSWC occurrence in 2005.

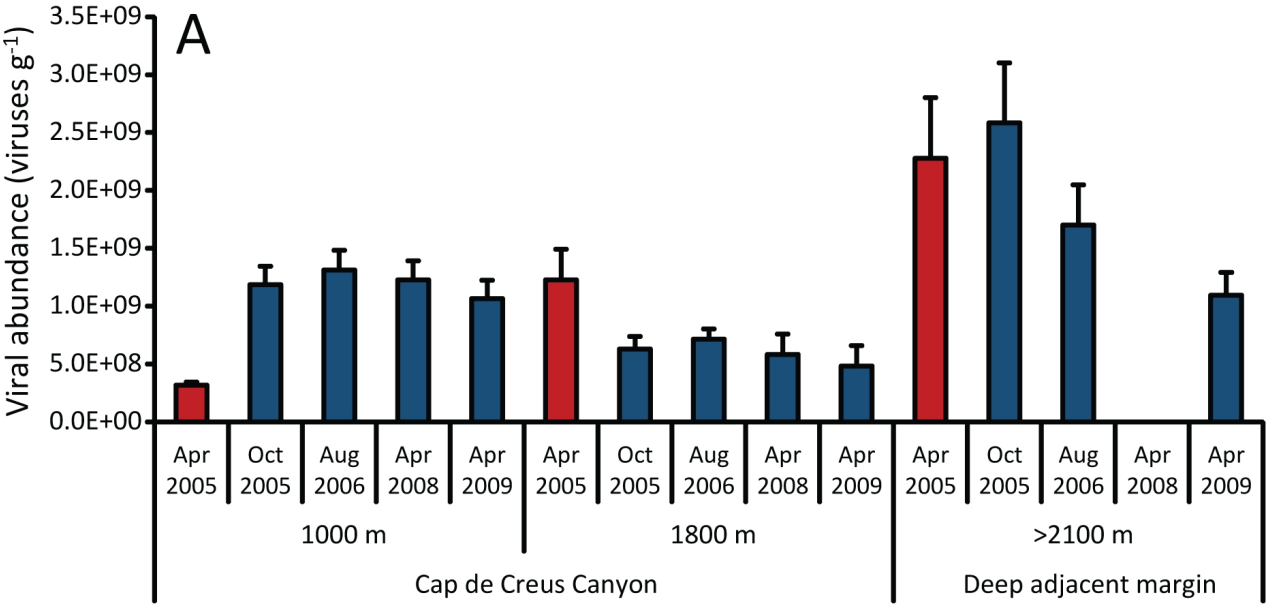
Figure 3. Spatial and temporal changes of prokaryotic abundance (A), biomass (B) and heterotrophic C production (C) in surface sediments of the Cap de Creus Canyon and in the deep adjacent margin. Reported are mean and standard deviations. Red columns indicate intense DSWC occurrence in 2005.

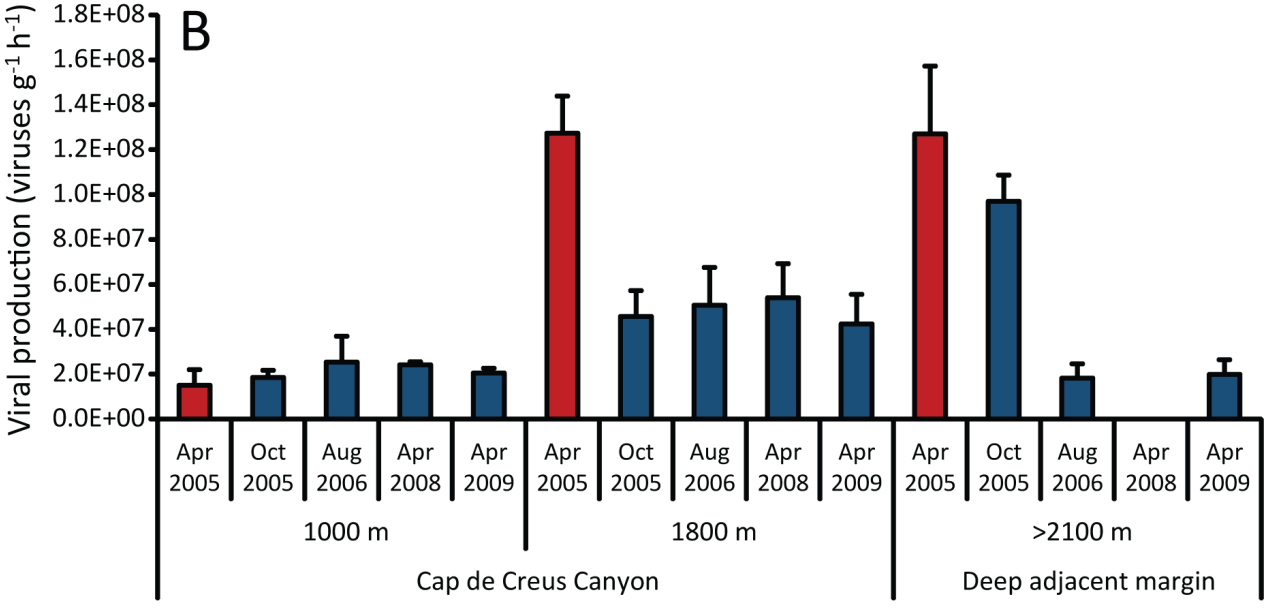
Figure 4. Spatial and temporal changes of viral abundance (A), production (B) and virus-induced prokaryotic mortality – VIPM (C) in surface sediments of the Cap de Creus Canyon and the deep adjacent margin. Reported are mean and standard deviations. Red columns indicate intense DSWC occurrence in 2005.

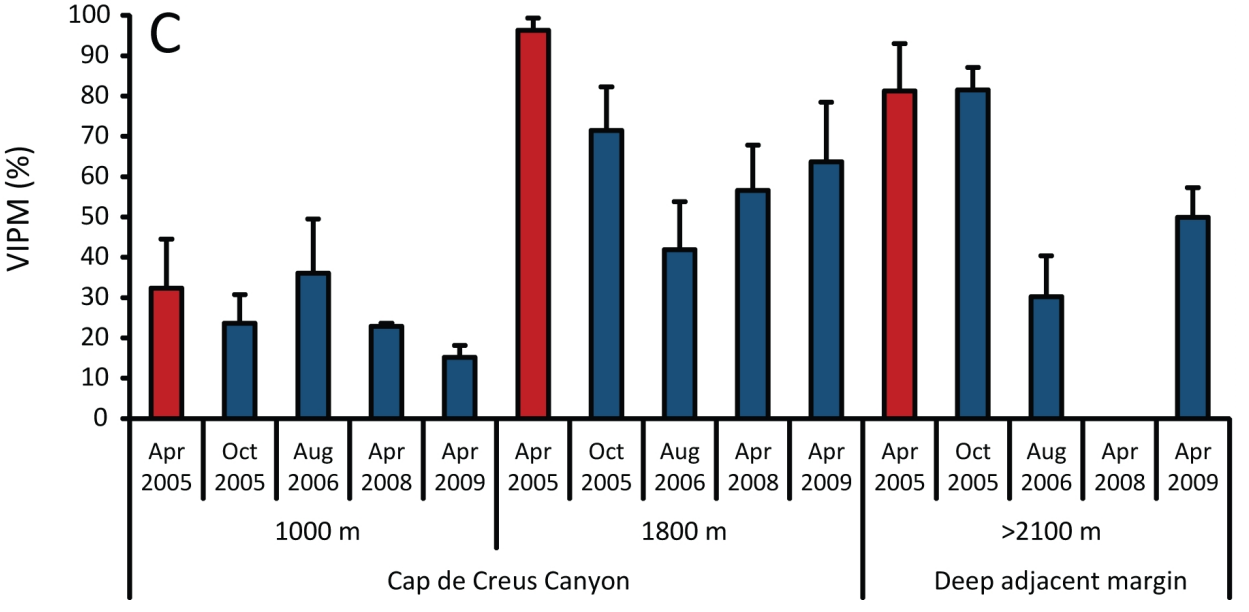

3°30'0"E
4°0'0"E
4°30'0"E
42°20'0"N
42°0'0"N
Lacaze-Duthiers Canyon
Cap de Creus Canyon
Sète Canyon
April 2005
October 2005
August 2006
April 2008
April 2009
April 2005
October 2005
August 2006
April 2008
April 2009
Deep margin
April 2005
October 2005
August 2006
April 2008
April 2009
200
600
1000
1600
2000


Phytopigments concentration (µgC g-1)
6.0E+01
5.0E+01
4.0E+01
3.0E+01
2.0E+01
1.0E+01
0.0E+00
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
1000 m
1800 m
>2100 m
Cap de Creus Canyon
Deep adjacent margin


A
Prokaryotic abundance (cells g-1)
1.6E+09
1.4E+09
1.2E+09
1.0E+09
8.0E+08
6.0E+08
4.0E+08
2.0E+08
0.0E+00
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
1000 m
1800 m
>2100 m
Cap de Creus Canyon
Deep adjacent margin
B
Prokaryotic biomass (µgC g-1)
6.0E+01
5.0E+01
4.0E+01
3.0E+01
2.0E+01
1.0E+01
0.0E+00
C
Heterotrophic C production (ngC g-1 h-1)
1.4E+02
1.2E+02
1.0E+02
8.0E+01
6.0E+01
4.0E+01
2.0E+01
0.0E+00


A
Viral abundance (viruses g-1)
3.5E+09
3.0E+09
2.5E+09
2.0E+09
1.5E+09
1.0E+09
5.0E+08
0.0E+00
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
1000 m
1800 m
>2100 m
Cap de Creus Canyon
Deep adjacent margin


B
Viral production (viruses g-1 h-1)
1.8E+08
1.6E+08
1.4E+08
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
1000 m
1800 m
>2100 m
Cap de Creus Canyon
Deep adjacent margin


C
VIPM (%)
100
90
80
70
60
50
40
30
20
10
0
Apr 2005
Oct 2005
Aug 2006
Apr 2008
Apr 2009
1000 m
1800 m
>2100 m
Cap de Creus Canyon
Deep adjacent margin