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# Ocean acidification effect on prokaryotic metabolism tested in two diverse trophic regimes in the Mediterranean Sea

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ABSTRACT

Notwithstanding the increasing amount of researches on the effect of ocean acidification (OA) on marine ecosystems, no consent has emerged on its consequences on many prokaryote-mediated processes. Two mesocosm experiments were performed in coastal Mediterranean areas with different trophic status: the summer oligotrophic Bay of Calvi (BC, Corsica, France) and the winter mesotrophic Bay of Villefranche (BV, France). During these experiments, nine enclosures (~54 $m^3$) were deployed: 3 unamended controls and 6 elevated $CO_2$, following a gradient up to 1250 μatm. We present results involving free-living viral and prokaryotic standing stocks, bacterial carbon production, abundance of highly active cells (CTC+), and degradation processes (beta-glucosidase, chitinase, leucine-aminopeptidase, lipase and alkaline phosphatase activities).

The experiments revealed clear differences in the response of the two prokaryotic communities to $CO_2$ manipulation. Only abundances of heterotrophic prokaryotes, viruses and lipase activity were not affected by $CO_2$ manipulation at both locations. On the contrary, the percent of CTC+ was positively correlated to $CO_2$ only in BC, concomitantly to a bulk reduction of [$^3$H]-leucine uptake. The other tested parameters showed a different response at the two sites suggesting that the trophic regime of the systems plays a fundamental role on the effect of OA on prokaryotes through indirect modifications of the available substrate.

Modified degradation rates may affect considerably the export of organic matter to the seafloor and thus ecosystem functioning within the water column. Our results highlight the need to further analyse the consequences of OA in oligotrophic ecosystems with particular focus on dissolved organic matter.



## 1. Introduction

Microbes play a major role within biogeochemical cycles in the ocean (Azam, 1998). Heterotrophic and mixotrophic prokaryotes are responsible for more than half of the primary production processing through the modification and utilization of organic macromolecules (Ducklow and Carlson, 1992). Natural and human-induced changes to the environment can result into alteration of microbial activities and consequently of biogeochemical cycling (e.g. Nogales et al., 2011). Nowadays, ocean acidification (OA) is an ongoing process with potential harmful consequences to the marine ecosystem and its resources (e.g. Cerrano et al., 2013; Gazeau et al., 2013). OA is defined as the ocean pH decrease due to the dissolution of atmospheric carbon dioxide. pH is a key parameter for life, especially for aquatic organisms. Cell metabolism is mediated by a wide range of enzymatic reactions that require an optimum pH range. Intracellular pH is maintained near neutrality in order to prevent the damage of acid- or alkali-labile molecules; on the other hand, in a pH-changing environment extracellular enzymes are potentially affected by OA (Piontek et al., 2010 and references therein).

Aquatic prokaryotes utilize exoenzymes to hydrolyse organic matter in order to obtain smaller molecules to be taken up and used in several metabolic pathways (Chróst, 1992). This ability to 'digest'

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a wide array of organic material, from simple dimers to complex particles, has led to the definition of bacteria as 'swimming stomachs' (as referenced in Azam, 1998). Expression of glucosidases, proteases, lipases, phosphatases and other hydrolytic enzymes, in fact, allows the breakdown of organic matter and the ultimate assimilation of monosaccharides, amino acids, orthophosphates and other simple compounds into the cell. The hydrolysis-uptake coupling determines the efficiency of microbial communities and their role of master recycler (Buchan et al., 2014) in ecosystem functioning. Prokaryotes rework, utilize and produce dissolved organic matter; under strong predation by bacterivores, prokaryotic biomass is channelled to higher trophic levels. When grazing pressure is limited, their standing stocks are mainly controlled by viral lysis and dissolved organic matter (DOM) availability where DOM consumption and production are maintained in the so called 'microbial loop' (Azam et al., 1983). Therefore, the mineralization of organic material in the surface ocean influences the carbon fluxes in the ecosystem i.e. sinking to the deep ocean vs. transfer to higher trophic levels (Chisholm, 2000).

The effect of OA on marine prokaryotes has been studied in the last decade using several approaches, considering different acidification protocols, different volumes of experimental enclosures and different secondary amendments (e.g. nutrient addition). Researches highlighted a general lack of consensus among the obtained results; in fact, disentangling the role of lowered pH, of increased dissolved inorganic carbon, and indirect modifications of the organic matter pool is challenging. The set-up of experiments in systems with different trophic status further complicates this picture. In the first reported study on this topic, Grossart et al (2006) observed modifications in exoenzymatic activities (increase of protease, and, to a lesser extent, α- and β-glucosidase activities) in relation to OA and interpreted these results as an indirect consequence of enhanced particles production by phytoplankton. Proteolytic activities were found to be either enhanced (Piontek et al., 2013; Endres et al., 2014), unaffected (Maas et al., 2013) or inhibited (Yamada and Suzumura, 2010) by increased partial pressure of $CO_2$ ($pCO_2$)/decreased pH, and similar results have been obtained when testing lipase activity (Yamada and Suzumura, 2010; Maas et al., 2013). Limited variations have been reported for alkaline phosphatase (Tanaka et al., 2008; Yamada and Suzumura, 2010; Maas et al., 2013) and chitinase activities (Maas et al., 2013). Polysaccharide degradation was generally enhanced by acidification (Piontek et al., 2010, 2013; Maas et al., 2013) and the tendency is to link such result to phytoplankton and organic matter dynamics (e.g. Arnosti et al., 2011).

Since degradation processes increase the availability of utilizable organic matter, bacterial heterotrophic carbon production (uptake of monomers) might be affected positively or negatively by an increased $CO_2$ level. The general findings of increased primary production under elevated $CO_2$ conditions suggest that the highest amount of organic carbon (in the form of dissolved organic carbon, transparent exopolymeric particles, biomass and particulate detritus) in $CO_2$-treated enclosures would fuel heterotrophic production and prokaryotic growth (Grossart et al., 2006; Piontek et al., 2013). However, different scenarios have been depicted by other researchers. Motegi et al. (2013) reported a decrease in thymidine uptake rates under nutrient enrichment at different $pCO_2$ levels, suggesting the potential role of several parameters other than $CO_2$, such as dissolved organic matter composition.

Furthermore, the fate of viruses in an acidified ocean is not clearly unveiled as well. Rochelle-Newall et al. (2004), found no difference in viral abundances between controls and $CO_2$-amended mesocosms, whereas other authors detected a $pCO_2$ impact on viruses infecting some phytoplankton species (Larsen et al., 2008; Brussaard et al., 2013). Notably, such findings have to be related to the mutual dependence of viruses on their hosts (metabolism and diversity).

Most of the mesocosms studies aimed at investigating the impact of increased $CO_2$ on marine plankton have been carried out in mesotrophic or eutrophic waters (naturally or after nutrient addition), and there currently is a lack of information concerning oligotrophic (low-nutrient low-chlorophyll) environments that represent a very large portion of the ocean.

To date, no report on Mediterranean microbial community response to ocean acidification is available. For this reason, two mesocosm experiments have been performed in coastal waters located in the western basin of the Mediterranean Sea in summer 2012 (Bay of Calvi, Corsica, France) and late winter 2013 (Bay of Villefranche, France). The goal of these comparative studies was to understand the response of planktonic communities to increased $pCO_2$ in low nutrient-low chlorophyll areas. We present our results in the light of the differences emerging from seasonality and site-specific trophic status. The present study focuses on the effect of OA on free-living prokaryotic and viral standing stocks, organic matter degradation processes (beta-glucosidase, alkaline phosphatase, lipase, chitinase and leucine aminopeptidase) and bacterial carbon production. Our aim was to answer the following questions: 1) Are prokaryotic growth and viral dynamics affected by high-$pCO_2$ in low nutrient-low chlorophyll areas? 2) Is organic matter degradation and utilization altered by ocean acidification in oligotrophic coastal Mediterranean areas?

## 2. Materials and methods

### 2.1. Experimental set-up

Two mesocosm experiments were setup in the Northwestern Mediterranean Sea. The first experiment took place in the Bay of Calvi (BC; NW Corsican coast, France) in summer 2012 and the second in the Bay of Villefranche (BV; South-Eastern France) in late winter 2013. Both locations had a water column depth of 25 m. Briefly, nine ~50,000 L-mesocosms (2.3 m in diameter and 12 m in height), made of 500 μm thick films of polyethylene mixed with vinyl acetate and covered with UV-transparent ETFE roofs, were deployed on June 17th 2012 and on February 14th 2013 in BC and BV respectively (for a detailed description of mesocosm materials, setup, acidification and experimental timelines see Gazeau et al., 2017a). Briefly, on June 20th 2012 (BC) and February 17th 2013 (BV), after the closing of the bags, six mesocosms were amended with $CO_2$-saturated seawater over 4 days in order to obtain a concentration range from ambient level to 1250 μatm (P1–P6), thus covering the projected range of $pCO_2$ for the end of the century following different emission scenarios (RCP2.6–RCP8.5; IPCC 2013). Three unamended mesocosm were used as controls (C1–C3) with $pCO_2$ levels of 450 and 350 μatm in BC and BV, respectively. In BC, the six targeted elevated $pCO_2$ levels were P1: 550, P2: 650, P3: 750, P4: 850, P5: 1000 and P6: 1250 μatm. In BV, the levels were P1: 450, P2: 550, P3: 750, P4: 850, P5: 1000 and P6: 1250 μatm. Mesocosms were anchored in clusters of 3, each cluster containing one control mesocosm accompanied by a medium and a high $pCO_2$ level (cluster 1: C1, P1, P4; cluster 2: C2, P2, P5 and cluster 3: C3, P3, P6). On June 24th 2012 and February 22nd 2013 in BC and BV, respectively, once targeted $pCO_2$ levels were reached, the experiment started (day 0). The experiment lasted 20 days in BC, but as a storm caused non-repairable damages to the bags on March 7th in BV, this latter experiment had to be terminated after 12 days.

Before sunrise (4.00 a.m. in BC and 5.00 a.m. in BV, local times), depth-integrated samples (from surface to 10 m) were collected in each mesocosm by means of 5 L Hydro-Bios integrated bottle samplers and processed within 1 h. Samples for heterotrophic