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Ocean acidification and viral replication cycles: Frequency of lytically infected and lysogenic cells during a mesocosm experiment in the NW Mediterranean Sea

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ABSTRACT

The frequency of lytically infected and lysogenic cells (FLIC and FLC, respectively) was estimated during an *in situ* mesocosm experiment studying the impact of ocean acidification on the plankton community of a low nutrient low chlorophyll (LNLC) system in the north-western Mediterranean Sea (Bay of Villefranche, France) in February/March 2013. No direct effect of elevated partial pressure of CO_2 (pCO_2) on viral replication cycles could be detected. FLC variability was negatively correlated to heterotrophic bacterial and net community production as well as the ambient bacterial abundance, confirming that lysogeny is a prevailing life strategy under unfavourable-for-the-hosts conditions. Further, the phytoplankton community, assessed by chlorophyll *a* concentration and the release of >0.4 μm transparent exopolymeric particles, was correlated with the occurrence of lysogeny, indicating a possible link between photosynthetic processes and bacterial growth. Higher FLC was found occasionally at the highest pCO_2-treated mesocosm in parallel to subtle differences in the phytoplankton community. This observation suggests that elevated pCO_2 could lead to short-term alterations in lysogenic dynamics coupled to phytoplankton-derived processes. Correlation of FLIC with any environmental parameter could have been obscured by the sampling time or the synchronization of lysis to microbial processes not assessed in this experiment. Furthermore, alterations in microbial and viral assemblage composition and gene expression could be a confounding factor. Viral-induced modifications in organic matter flow affect bacterial growth and could interact with ocean acidification with unpredictable ecological consequences.

1. Introduction

The rise in CO_2 emissions due to human activities, such as fossil fuel burning, is currently well established (Cubasch et al., 2013). The oceans absorb ~25% of CO_2 emissions, leading to an increase in the partial pressure of CO_2 (pCO_2), dissolved inorganic carbon and bicarbonate ion concentrations as well as a decrease in carbonate ion concentrations and pH (the so-called ocean acidification; Gattuso and Hansson, 2011). These changes in carbonate chemistry are hypothesized to significantly affect biological processes (e.g. Riebesell and Tortell, 2011) and the cycles of nutrients (Hutchins et al., 2009). Predicting the effects of ocean acidification on oceanic biogeochemistry has inherent difficulties as several biochemical characteristics could be indirectly influenced, such as the quality and quantity of dissolved and particulate organic matter (Grossart et al., 2006).

The physiology of several marine species has been found to be affected by a decline in pH (e.g. Kroeker et al., 2013). Numerous hypotheses have been proposed in order to predict and explain the responses of these species (Beardall et al., 2009), particularly of primary producers, based on their carbon acquisition pathways (Riebesell and Tortell, 2011). However, most research studies have

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focused on single strains and used short-term incubations. As a result, the impact of ocean acidification at the community level, where species interactions are complex, is rather unclear (Riebesell and Gattuso, 2014).

In situ large seawater enclosures provide the opportunity to study multiple levels of microbial assemblages. They are a valuable experimental tool that bridges natural and laboratory-produced observations, and have been extensively used in order to answer specific ecological questions (Taub, 1997). In large-scale mesocosm experiments, the effect of ocean acidification on microbial assemblages has been assessed (e.g. Riebesell et al., 2008, 2013), but viruses - the common denominator of all planktonic community members - have received the least attention (Larsen et al., 2008; Danovaro et al., 2011; Brussaard et al., 2013; Carreira et al., 2013).

As a result, it is uncertain if, in the future, viral infection and ocean acidification will interact and how these two processes could ultimately shape plankton community dynamics and the associated biological carbon pump (Danovaro et al., 2011). Enhanced bacterial respiration and viral activity may either reduce the efficiency of the biological pump by favouring the microbial loop and limiting carbon transfer to higher trophic levels and the deep ocean (Suttle, 2007) or have the opposite effect by promoting aggregation and accelerating sinking rates (Brussaard et al., 2008).

An important mechanism for particle aggregation is the formation of transparent exopolymeric particles (TEP) by phytoplankton. TEP formation has proven to be elevated under increased CO_2 concentrations (e.g. Engel, 2002, 2004). TEP are highly enriched in carbon relative to nitrogen and phosphorus molecules and their exudation may alter viral life cycles as they are attachment sites that favour bacteria. In a similar manner, potential changes in the flow and availability of nutrients due to ocean acidification may alter the composition and metabolic rates of bacteria, thus viral life cycles too (Danovaro et al., 2011).

Viruses are the smallest oceanic entities that interact with all planktonic components and impact global biogeochemical processes (Wommack and Colwell, 2000). They alter nutrient cycling through the release, uptake and remineralization of organic and inorganic material during lysis events (Zubkov et al., 2003; Poorvin et al., 2004; Bonilla-Findji et al., 2008), and they affect the plankton community growth and structure by causing significant levels of bacterial mortality and elevated richness (Thingstad, 2000) by preferentially infecting/lysing either closely related strains or distant ones (e.g. Sullivan et al., 2003). The genetic composition and evolution of viruses and hosts are also affected, as some viruses can incorporate host genes in their genome and mediate horizontal gene transfer (Sullivan et al., 2005).

Two main viral life strategies have been defined and play a key role in the above-mentioned processes. On one hand, rapid replication and immediate host-cell death define the lytic cycle. On the other hand, incorporation of the viral genome in the bacterium in a quiescent stage for a variable number of generations characterizes the lysogenic cycle. When lysogenic bacteria are exposed to a physical or chemical inducing agent, activation of the incorporated viral genomes can occur. It is a temporally and spatially variable life strategy (e.g. McDaniel et al., 2002; Maurice et al., 2010) that has been studied in lagoons (e.g. Maurice et al., 2013), freshwater (e.g. Thomas et al., 2011) and several types of marine environments (e.g. Bongiorni et al., 2005; Bettarel et al., 2008). Both lytic and lysogenic viral life strategies have evolved in response to ambient environmental status (McDaniel and Paul, 2005). Lysogeny in particular is assumed to be a beneficial life strategy for both hosts and viruses under unfavourable conditions, in contrast to lysis that prevails mostly under nutrient-replete conditions (Weinbauer et al., 2003). For instance, during periods of low host abundance and overall low system productivity, lysogeny provides increased hosts' fitness through protection against environmental stress (e.g. UV radiation, Bettarel et al., 2008) as well as through immunity to super-infection (Long et al., 2008).

The present experiment focused on the importance of lysogeny as a key indicator of environmental status under the impact of ocean acidification. Both lytic viral production and the frequency of lysogeny were measured in response to increased pCO_2 based on a viral reduction approach. The frequency of lytically infected and lysogenic cells (FLIC and FLC, respectively) was estimated during a 12-day *in situ* mesocosm experiment carried out in the Bay of Villefranche (France), in a typical Low Nutrient Low Chlorophyll (LNLC) system of the north-western (NW) Mediterranean area. During this experiment, nine mesocosms were used, among which six were enriched with CO_2-saturated water to obtain a pCO_2 gradient ranging from ambient levels (~356 μatm) to 1327 μatm. Sub-samples for the determination of FLIC and FLC were taken from two control mesocosms (C1 and C3) that were not manipulated with CO_2, as well as from three CO_2-enriched mesocosms (P1, P5 and P6; corresponding to initial pCO_2 conditions of 595, 1174 and 1327 μatm, respectively) at three time points (experimental days 1, 5 and 9). The main goal of this study was to determine if and how pH and ambient environmental conditions affect the patterns of the two viral replication strategies in natural microbial assemblages. We hypothesized that lysis and lysogeny are governed by the production rates of the hosts and the productivity status of the environment, which may be affected by the pH manipulation. We explored this hypothesis by assessing several biotic and abiotic parameters, including the (1) seawater pH, (2) abundance of viruses, heterotrophic and autotrophic bacteria, (3) concentrations of chlorophyll *a* (Chl) and TEP as indicative factors of the phytoplankton community, and (4) heterotrophic and autotrophic bacterial, and net community production rates (BP, PP and NCP, respectively).

2. Materials and methods

2.1. Experimental set up

A mesocosm experiment was performed between 21st February and 5th March 2013 in the Bay of Villefranche, which is located in the northern part of the Ligurian Sea (NW Mediterranean). The experimental set-up consisted of nine cylindrical enclosures, which were made only with plastic materials (polyethylene mixed with vinyl acetate). The cylindrical part of the mesocosms was 2.3 m in diameter and 12.5 m in height, resulting in a theoretical final water volume of ca. 50 m^3. Mesocosms were covered with UV-transparent ETFE (ethylene tetrafluoroethylene) roofs (except during samplings) in order to avoid atmospheric deposition. On February 15th, 2013, the mesocosms were deployed in the Bay of Villefranche. The bottom ends of the mesocosm bags were lowered to a depth of 10 m below the surface to enclose the plankton community with minimal disturbance to the water column. A mesh of 5 mm was attached to the bottom to exclude large organisms. Mesocosms were left open for two days to insure a proper homogenization of the communities entrapped. After two days, the nets were removed and the bottoms of the mesocosms were closed by divers. In order to minimize the stress induced by the addition of large quantities of acidified water, the acidification of the mesocosms was performed over four days. Six of them were modified in terms of pCO_2 (referred to as perturbed mesocosms or P) following a targeted gradient: 450 (P1), 550 (P2), 750 (P3), 850 (P4), 1000 (P5) and 1250 (P6) μatm, while the triplicate control mesocosms (C1, C2 and C3) presented an initial pCO_2 of 356 ± 14 μatm. Higher pCO_2 levels in the perturbed mesocosm were reached by adding various volumes of CO_2-saturated seawater. Saturated seawater was

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