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"Changes in the microbial community based on seawater pH variations"

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CONTENTS

ABSTRACT

Ocean acidification is an effect of the rise in atmospheric CO_2, which causes a reduction in the pH of the ocean and generates a number of changes in seawater chemistry and consequently potentially impacts seawater life.

The effect of ocean acidification on metabolic processes (such as net community production and community respiration and on particulate organic carbon (POC) concentrations was investigated in summer 2012 at Cap de la Revellata in Corsica (Calvi, France). Coastal surface water was enclosed in 9 mesocosms and subjected to 6 pCO_2 levels (3 replicated controls and 6 perturbations) for approximately one month. No trend was found in response to increasing pCO_2 in any of the biological and particulate analyses. Community respiration was relatively stable throughout the experiment in all mesocosms, and net community production was most of the time close to zero. Similarly, POC concentrations were not affected by acidification during the whole experimental period. Such as the global ocean, the Mediterranean Sea has an oligotrophic nature. Based on present results, it seems likely that seawater acidification will not have significant effects on photosynthetic rates, microbial metabolism and carbon transport.

1. INTRODUCTION

The ocean is a significant sink for anthropogenic carbon dioxide (CO_2) and has an important role in regulation of the CO_2 atmospheric concentration by physical, chemical and biological processes. The concentration of CO_2 in the atmosphere has increased from 172-300 parts per million by volume (ppmv) in the pre-industrial era (Luthi et al., 2008), to 387 ppmv in 2009. The rate of increase was 1.0% yr^{-1} in the 1990s and reached 3.4% yr^{-1} between 2000 and 2008 (Le Quéré et al., 2009). Future levels of atmospheric CO_2 may reach 1020 ppmv in 2100 (IPCC, 2007).

The atmospheric CO_2 is dissolved in seawater, reacts and changes its chemical proprieties (Zeebe & Wolf-Gladrow, 2001). It is convolved in a complex reaction of balance between dissolved CO_2, bicarbonate and carbonate ions. Ocean acidification refers to a reduction in the pH of the ocean over an extended period, caused mainly by the uptake of anthropogenic CO_2 from the atmosphere. Mean surface ocean pH expressed on the total hydrogen ion scale (pH_T) has decreased from approximately 8.2 to 8.1 between pre-industrial period and the 1990s and may decrease by 0.3 – 0.4 unit (Caldeira & Wickett, 2003), reaching 7.8 in 2100 (Gattuso & Lavigne, 2009).

These changes in marine chemistry could lead to changes in carbon production and consumption (Riebesell & Tortell, 2011), and, therefore, to changes in oxygen production and consumption. Net community production (NCP) is defined as the balance between gross primary production (GPP) and community respiration (CR). NCP thus describes the net metabolism of the ecosystem. A positive NCP indicates that more organic carbon is produced than respired, so-called net autotrophy, while negative NCP indicates that respiration exceeds primary production, conducting to net heterotrophy.

The balance between photosynthetic carbon production and consumption of organic carbon in the ocean's surface layer is important to understand the ocean's role in the global cycle, so-called "microbial loop" (Azam *et al.,* 1983; Cho & Azam, 1988; Azam & Malfatti, 2007). Marine phytoplankton plays an important role in the carbon cycle, being responsible for about half of the global primary production (Field *et al.,* 1998). A large portion of organic carbon produced by photosynthesis is remineralized by respiration (del Giorgio & Duarte, 2002). Heterotrophic prokaryotes (hereafter "bacteria") can consume a significant

fraction of primary production in pelagic systems (Cole *et al*., 1988; Ducklow & Carlson, 1992). Mineral nutrients (e.g. N, P) can be a limiting factor of growth or organic carbon production by phytoplankton and bacteria.

Primary production is based on CO_2 as the main substrate, and since the CO_2-binding enzyme RuBisCO has a low affinity for its substrate (Badger *et al.,* 1998), an increase in seawater pCO_2 was hypothesized to stimulate phytoplankton PP (Riebesell *et al.,* 2000; Schippers *et al*., 2004; Rost *et al.,* 2008). The effect of seawater carbonate chemistry on photosynthesis thereby depends strongly on the presence and characteristics of cellular CO_2-concentrating mechanisms (CCMs; Rost *et al.,* 2003; Giordano *et al.,* 2005). Phytoplankton species that are able to enhance their CO_2 supply by CCMs may exhibit no or minimal sensitivity to CO_2 enrichment (Raven & Johnston, 1991; Rost *et al.,* 2003; Giordano *et al.,* 2005, Reinfelder, 2011).

The effects of increasing pCO_2 on production and respiration of pelagic plankton have been studied on single-species in laboratory cultures and on semi-natural communities in field mesocosms. Primary production measured by ^{14}C fixation or production of particulate organic carbon (POC) at elevated pCO_2 is enhanced (Hein & Sand-Jensen, 1997; Riebesell *et al.*, 2000; Zondervan *et al.*, 2001; Schippers *et al*., 2004; Leonardos & Geider, 2005; Egge *et al.,* 2009; Borchard *et al.*, 2011, Engel, 2002, Engel *et al*., 2012), decreased (Sciandra *et al.,* 2003), or shows no significant difference compared to the control (Tortell *et al.,* 2002; Delille *et al.,* 2005; Langer *et al.,* 2006). Measurement of primary production based on ^{14}C fixation or POC production are relatively numerous but few studies have examined the metabolic balance (i.e. NCP, CR, and GPP) of planktonic communities based on changes of dissolved oxygen (DO) concentration at different pCO_2 levels. The oxygen-based NCP measurement has shown a significant decrease in NCP of *Emiliania huxleyi* at elevated pCO_2 in a N-limited chemostat culture (Sciandra *et al*., 2003), and insignificant changes in NCP of semi-natural plankton community at different pCO_2 levels in mesocosm experiments (Delille *et al.,* 2005; Egge *et al*., 2009, Tanaka *et al.,* 2012).

The objective of the present study was to investigate the effect of ocean acidification on the balance between GPP and CR (i.e. NCP) and on the particulate organic carbon production of a plankton community in the Mediterranean Sea. In summer 2012, a multidisciplinary experiment was conducted for about one month using free-floating mesocosms deployed at Cap de

la Revellata in Corsica (Calvi, France), as part of the MedSeA (Mediterranean Sea Acidification in a changing climate) project. A series of chemical, biogeochemical, biological, and physiological parameters were measured during this experiment. We have analyzed NCP, CR, and GPP based on changing concentrations of dissolved oxygen in incubation bottles, and POC concentrations together with other related chemical and biological parameters.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL SETUP AND SAMPLING

Nine mesocosms (52 m^3, 15 m deep) were deployed and moored in the bay on June 20th (Figure 1). Three mesocosms served as controls (pCO_2 ~ 450 µatm), and in the six others various volumes of CO_2-saturated seawater were added in order to reach and the following levels: 550, 650, 750, 850, 1000, 1250 µatm. The addition of CO_2-saturated water has been performed gradually over 4 days.

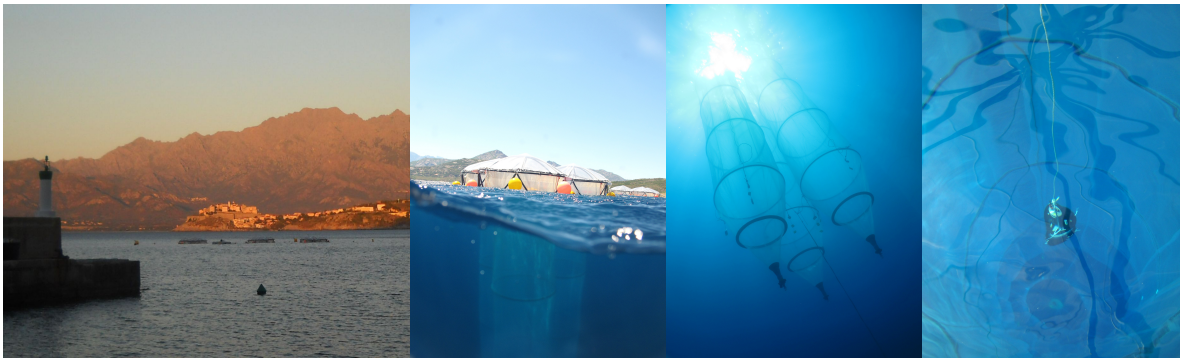

Figure 1. The bay of Calvi, mesoscosms and depth-integrated water sampler.

During the experimental period (June 24th – July 15th), depth-integrated water sampling and CTD profiles (0 - 10 m) were performed daily in each mesocosm using, respectively, three Hydrobios integrated water samplers (volume: 5 L) and a Sea Bird Electronics (SBE) 19plusV2 technology with additional sensors for fluorimetry, pH and dissolved oxygen. Samples for the measurement of community metabolism were collected before sunrise (4:00am - 5:30am) every second day, whereas those dedicated to carbonate chemistry, nutrients and particulate matter measurements were collected in the morning (8:30am – 10:30am) on a daily basis. In total, 10 samplings have been performed for community metabolism (June 24th – July 14th), while 21 samplings have been performed for carbonate chemistry, particulate matter and nutrients that ended on July 16th.

2.2. CARBONATE CHEMISTRY

2.2.1. DISSOLVED INORGANIC CARBON (DIC, C_T) MEASUREMENTS

C_T was measured daily by S. Alliouane and F. Gazeau (Laboratoire d'Océanographie de Villefranche, INSU-CNRS, Villefranche Sur Mer Cedex,

France) on the AIRICA (Automated Infra Red Inorganic Carbon Analyser) and were performed, at 25°C, on 1200 µL samples directly poisoned after sampling with $HgCl_2$. For calibration, 1100, 1200 and 1300 µL samples of the batch 117 (Certified Reference Material from A. Dickson. S = 33.503, C_T = 2009.99 µmol kg^{-1}, Total alkalinity (A_T) = 2239.18 µmol kg^{-1}) were measured.

2.2.2. TOTAL ALKALINITY (A_T) MEASUREMENTS

A_T was measured in the experimental samples using a Metrohm Titrando titrator following the procedure described in Dickson *et al.,* 2007 (SOP 3b). This parameter was measured daily from 24th June to 27th June and every second day from 27th June to 16th July because of its low variability. A_T measurements were performed on triplicate 50 mL samples at 25°C. Samples have been filtered onto GF/F and poisoned directly after filtration with $HgCl_2$. The electrode from the Metrohm Titrando titrator was calibrated every second day on the total scale using TRIS buffer solutions with a salinity of 35.0 (provided by A. Dickson).

The carbonate chemistry was calculated with the R package "seacarb", using C_T and A_T as well as temperature and salinity (based on integrated CTD profiles performed daily). In order to take into account the standard deviation of the measured parameters during the calculation of the carbonate chemistry parameters, a Monte-Carlo procedure was applied.

2.3. NUTRIENTS

The nutrients were measured daily by L. Michel (Stareso Research Station, Calvi, France) and S. Gobert (Laboratory of Oceanology, University of Liège, Liège, Belgium). Samples were directly frozen at -28 °C (for nitrites + nitrates, ammonium and phosphates) or kept at 4 °C (for silicates). No filtration was performed. Nutrient concentrations were determined spectrophotometrically, using a SKALAR continuous flow automated analyzer, following the methods of Strickland & Parson (1972) and Grasshoff *et al.* (1999), modified for oligotrophic seawater. Calibrations were performed before each analysis session. Analytical accuracy of data, checked using MOOS-2 certified reference material and internal blanks of nutrient exhausted seawater is typically between 0.02 and 0.05 µM.

2.4. CARBON AND NITROGEN PARTICULATE ANALYSIS

Two subsamples of about 2 L each were filtered on 25 mm glass fiber filters (Whatman GF/F) precombusted at 450 °C for 4 h. The filters were stored at -20 °C. The filters were folded and inserted in a tin capsula and analysed in CHNS-O. The filter for particulate organic carbon (POC) analysis was previously treated with the addition of HCl 1 N to remove the carbonate and then dried at 60 °C for about 1 h (Lorrain *et al.,* 2003).

The chemical elements were measured using an elemental analyser CHNO-S Costech mod. ECS 4010 by a high temperature oxidation at 980 °C, according to the method developed by Pella and Colombo (1973) and Sharp (1974). The sample and the capsule react with oxygen reaching temperatures of 1700-1800 °C. The combustion products pass through an oxidation column filled with Cr_2O_3 and a reduction column filled with copper wires in order to transform the carbon into CO_2, the hydrogen into H_2O and the nitrogen into molecular N_2. The water is absorbed on an anhydrous magnesium perchlorate trap.

The carrier gas, helium (He), brings the CO_2 and N_2 to a gas chromatographic separation column and to a TCD detector (Thermal Conductivity Detector). The TCD generates a signal, which is proportional to the amount of element in the sample. Known amounts of standard acetanilide (C_8H_9NO – Carlo Erba; Assay ≥ 99.5 %) were used to calibrate the instrument. The C and N sample concentrations are in µg C L^{-1} and µg N L^{-1} (ratio between the C or N amount resulting from the analysis and the filtered sea water volume after substracting the filter blanks).

2.5. NET COMMUNITY PRODUCTION, COMMUNITY RESPIRATION AND GROSS COMMUNITY PRODUCTION

After collection, water samples from each mesocosm were brought back to the shore and distributed into 15 biological oxygen demand (BOD) bottles (60 mL) by overflowing by 2-3 times the bottle volume. Five bottles were immediately fixed (T_0) with Winkler reagents, as described by Knap et al. (1996). The other 10 bottles were dedicated to the determination of net community production (NCP) and to community respiration (CR). Prior to filling, BOD bottles were washed with 5% HCl and rinsed thoroughly with Milli-Q water.

For each mesocosm, BOD bottles (x5) for NCP were incubated *in situ* at 5 m depth for 24 h at a mooring site located close to the mesocosms. BOD bottles used for the determination of CR (x5 for each mesocosm) were incubated in a laboratory incubator for 30 h, in which temperature was adjusted to the mean water temperature in the top 10 m on the day prior to sampling (21 - 24°C). Upon completion of the incubation, the bottles were immediately fixed with Winkler reagents.

DO concentrations were determined with an automated Winkler titration method using a potentiometric end-point detection (Titrando888, Metrohm). Reagents and standardizations were similar to those described by Knap *et al*. (1996). Rates of NCP and CR were determined by linear regression of DO against time (slope ± standard error: µmol O_2 L^{-1} d^{-1}). Gross primary production (GPP) was calculated as the difference between NCP and CR. The combined uncertainty of GPP (SE_{GPP}) was calculated using the standard error of NCP (SE_{NCP}) and CR (SE_{CR}).

$$SE_{GPP} = \sqrt{(SE_{NCP})^2 + (SE_{CR})^2}$$

The cumulative values of NCP, GPP and CR were calculated from the sum of every second day rates, in all mesocosms, during the whole experimental period. Every single values was multiplied by two, because of every second day sampling, but only the last values (14th July) was multiplied by four, because of a no-sampling day (12th July).

2.6. STATISTICAL ANALYSIS

In order to identify, for the various parameters, differences between the pCO_2 treatments, absolute deviations (AD(xi)) were calculated by subtracting from each observation (i.e. each mesocosm, Xi) the arithmetic mean of all observations ($\bar{x}$) at a specific time-point (t).

$$AD(x) = Xi - \bar{x}$$

Mean deviations (MD) were calculated for each mesocosm according to:

$$MD = \frac{1}{N} \Sigma ADx$$

With N being the number of observations and expressed as a relative value (%), according to Engel *et al.* (2012). Significance of the relationships between the mean deviations and average pCO_2 levels were tested using a Student t-test. Differences in data were considered significant for $p < 0.05$.

3. RESULTS

3.1. CARBONATE CHEMISTRY

All the parameters of the carbonate chemistry are presented in Table 1 (average ± SD during the course of the experiment) and in Figure 2.

Table 1. Parameters of carbonate chemistry, temperature and salinity. Average and standard deviation (between brackets is reported).

Mesocosm	Targeted pCO_2	Observed pCO_2	T	S	pH_T	A_T	C_T	Ωa	Ωc
	µatm	µatm	°C			µmol kg^{-1}	µmol kg^{-1}		
OUT	450	434 (2.65)	23.56	38.06	8.04 (0.002)	2537 (2.28)	2223 (1.02)	3.43 (0.02)	5.24 (0.02)
C1	450	429 (2.05)	23.63	38.04	8.05 (0.002)	2543 (1.56)	2225 (1.12)	3.47 (0.01)	5.30 (0.02)
C2	450	427 (1.98)	23.61	38.06	8.05 (0.002)	2545 (1.52)	2225 (0.99)	3.49 (0.01)	5.33 (0.02)
C3	450	429 (1.68)	23.63	38.03	8.05 (0.001)	2541 (1.09)	2223 (1.02)	3.47 (0.01)	5.30 (0.01)
P1	550	510 (2.24)	23.62	38.06	7.99 (0.002)	2544 (1.28)	2263 (1.11)	3.10 (0.01)	4.74 (0.02)
P2	650	589 (2.34)	23.63	38.07	7.94 (0.002)	2545 (0.83)	2294 (1.15)	2.81 (0.01)	4.30 (0.01)
P3	750	664 (3.12)	23.62	38.05	7.89 (0.002)	2542 (1.04)	2316 (1.20)	2.58 (0.01)	3.94 (0.01)
P4	850	751 (3.78)	23.63	38.06	7.84 (0.002)	2544 (1.15)	2341 (1.13)	2.37 (0.01)	3.61 (0.01)
P5	1000	837 (4.39)	23.63	38.04	7.81 (0.002)	2543 (1.23)	2359 (1.07)	2.20 (0.01)	3.36 (0.02)
P6	1250	1008 (6.39)	23.63	38.06	7.74 (0.003)	2544 (1.47)	2393 (1.09)	1.91 (0.01)	2.92 (0.02)

The starting pCO_2 values of perturbation mesoscosms (data not shown) were very close to the targeted values (see 2.1 section). pH and pCO_2 conditions in the control mesocosms were similar to those of the outside environment, following the same variations, throughout the whole experimental period (Figure 1). A_T was lower in the outside environment from July 4th to July 12th due to atmospheric events that mixed the water column. A_T increased gradually in all mesocoms, during the experimental period as a consequence of the water body isolation and subsequent evaporation.

Reached the targeted values, the pCO_2 decreased gradually in all "perturbed" mesocosms but more importantly in the high-CO2 mesocosms (P4, P5 and P6), during the experimental period as a consequence of CO_2 exchange between the water column and the atmosphere. Similarly, pH_T levels increased gradually in all "perturbed" mesocosms during the experimental period. CTD-pH profiles in the water column (data not shown) revealed that this parameter was perfectly vertically homogeneous during the whole experimental period.

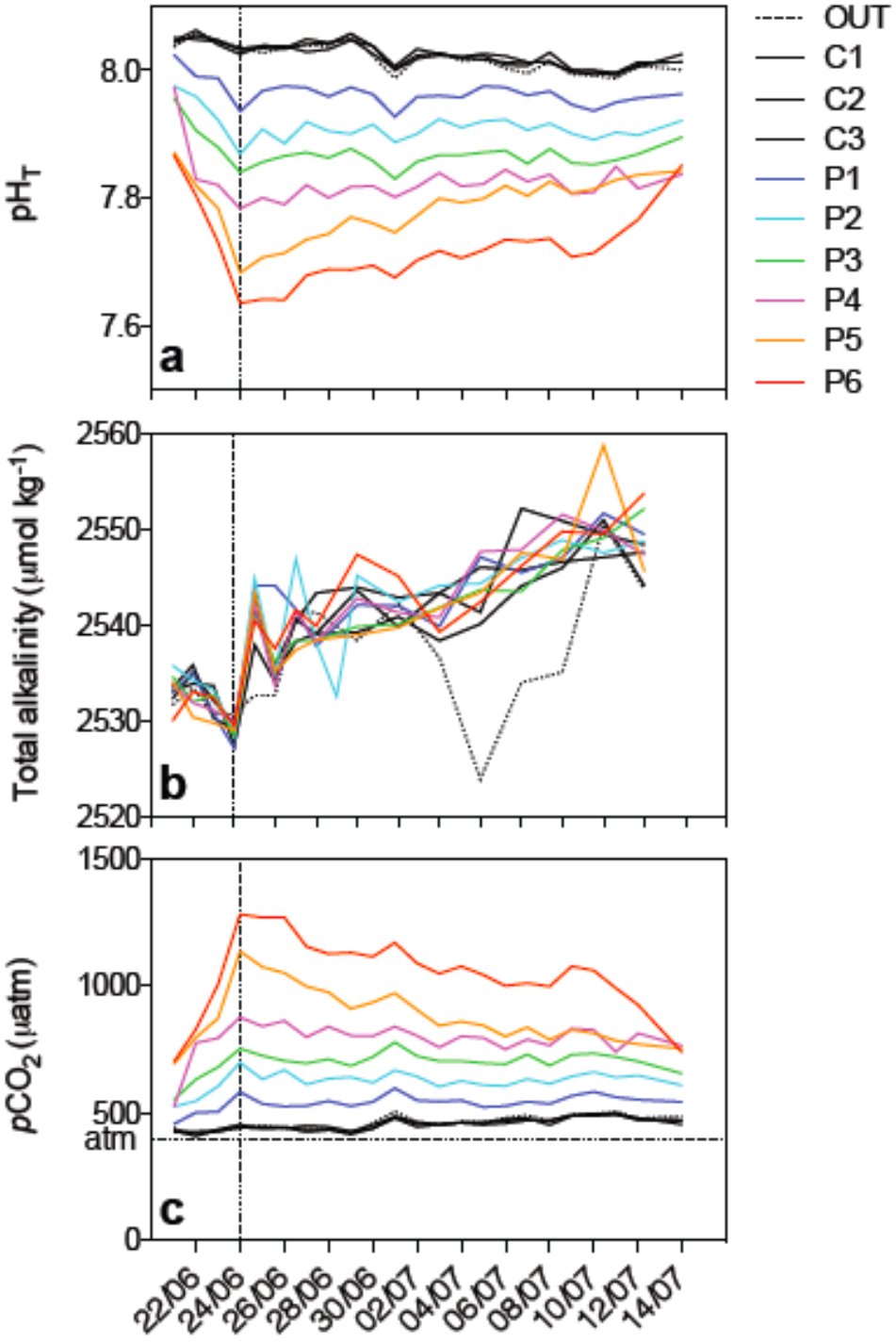

Figure 2. Trends of integrated pH_T, total alkalinity and pCO_2 values in all mesocosms (3 controls and 6 "low-pH" levels) as well as in the bay of Calvi (OUT) during the experimental period.

3.2. TEMPERATURE AND SALINITY

Mean temperature and salinity (± SD) are presented in Table 1. The variations in temperature were mainly related to weather conditions. Cyclical atmospheric events both enhanced mixing and convection processes, that homogenize the hydrological properties, and promoted calm and smooth conditions enhancing stratification (data not shown). Shifts between these two states were experienced, with three mixing events (June 22nd – 26th, July 3rd – 4th; July 11th – 16th) resetting stratified conditions. Water column integrated values showed that the heat content continuously increased until the last mixing event for all mesocosms and closely followed the outside dynamics (Figure 3.a).

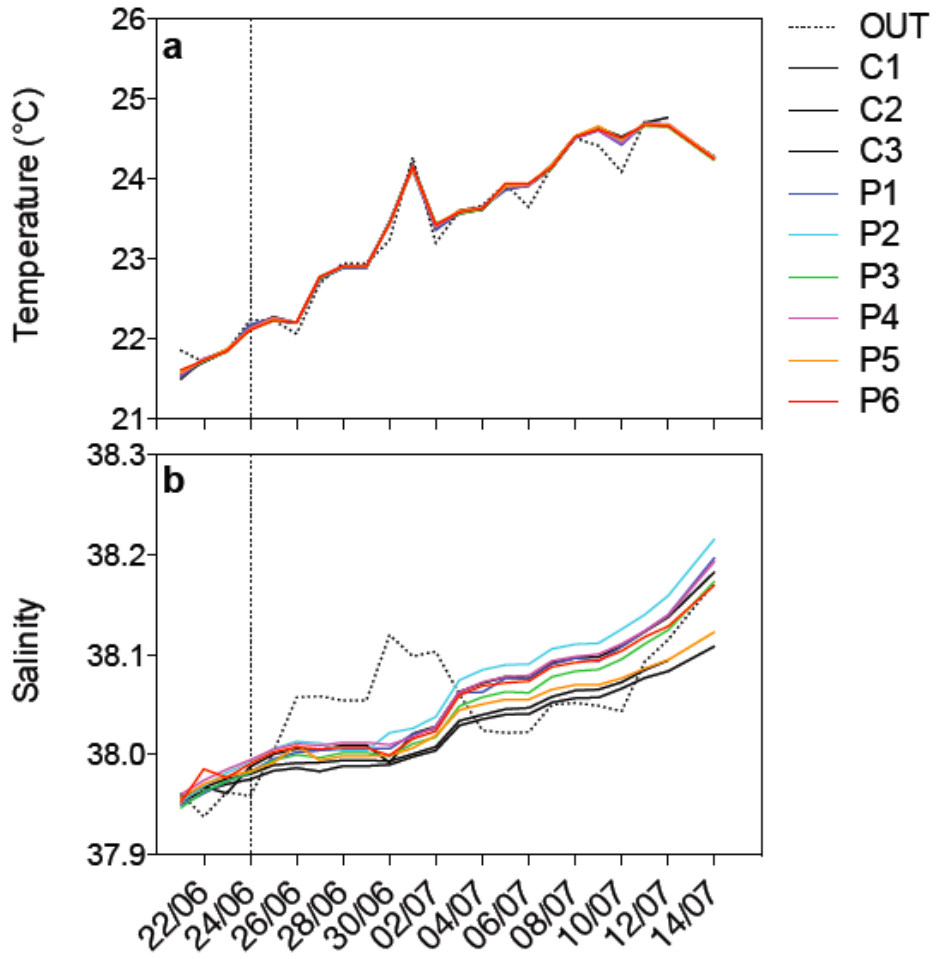

Figure 3. Water column integrated temperature (a) and salinity (b) evolutions during the course of the experiment in all nine mesocosms and in the bay of Calvi.

The variations in salinity in the mesocosms were less clearly related to outside conditions as the water masses were isolated from the surrounding environment. Salinity remained homogeneous in the water column in all

mesocosms (data not shown) and water-column integrated values are shown in Figure 3.b. While the variations in salinity outside the mesocosms were depending on circulation changes in the bay of Calvi (OUT), salinity increased during the course of the experiment in all mesocosms, with an accentuation at the end of the experiment. This can be explained by the presence of the cover topping mesocosms that artificially reduce sensible heat fluxes variations by decreasing temperature gradient and wind stress at air-sea interface. It has to be stressed that the dispersion in between mesocosms significantly increased at the end of the experiment. This can be due to external effects of dilution with respect to the wind burst of the last week.

3.3. FLUORESCENCE OF Chl a, NUTRIENTS AND OXYGEN CONCENTRATION

The water column integrated fluorescence signals in all nine mesocosms are shown in Figure 4.a.

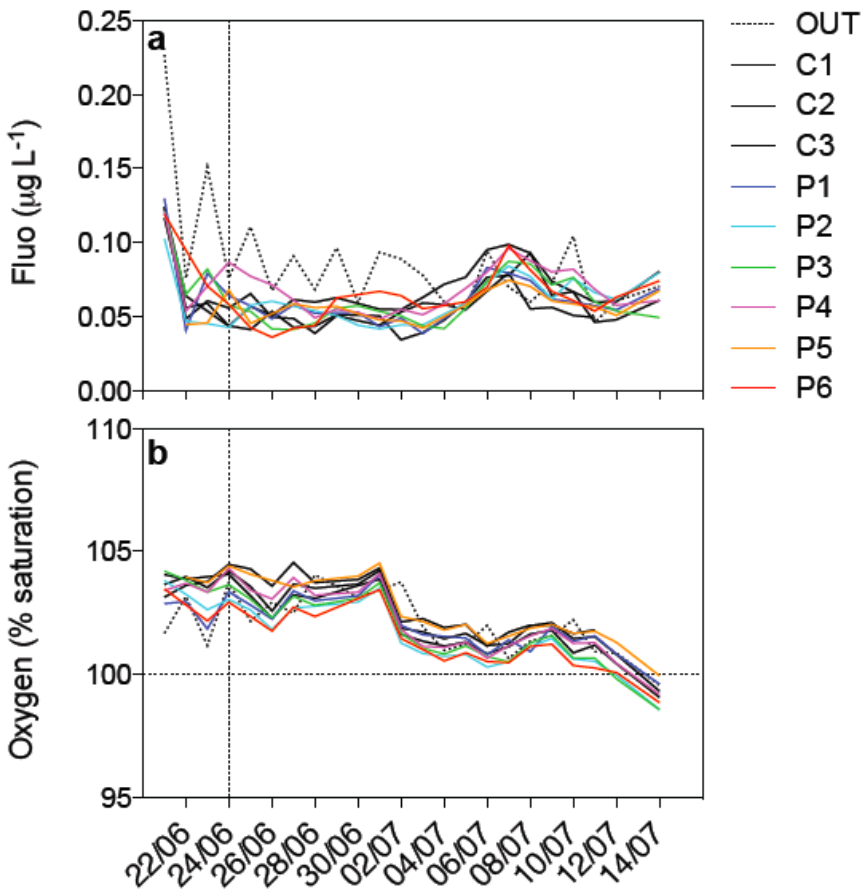

Figure 4. Integrated fluorescence Chl *a* (a) and oxygen saturation (b) evolutions during the course of the experiment in all nine mesocosms and in the bay of Calvi.

Very low chlorophyll a concentrations were observed with a decrease from 0.1 to 0.05 µg L^{-1} at the beginning of the experiment, and a peak up to 0.1 µg L^{-1} during the second mixing event (July 3rd – 4th) that followed a long stratification period. The deviations of chlorophyll a concentrations from the mean showed a not statistically significant relationship with pCO_2 (F-test, P > 0.05). These low values of fluorescence Chl_a can be explained by the presence of very low concentrations of nutrients, usually below the detection limits, at the exception of Silicates ($NO_3 \leq 0.1$ µmol L^{-1}; $NH_4^+ < 0.5$ µmol L^{-1}; $PO_4 <$ 0.07 µmol L^{-1}; Si < 2 µmol L^{-1}). Silicate concentrations showed a decline in all mesocosms from the beginning to the end of the experiment (Figure 5). The deviations from the mean showed a not statistically significant relationship with pCO_2 effect (F-test, P > 0.05).

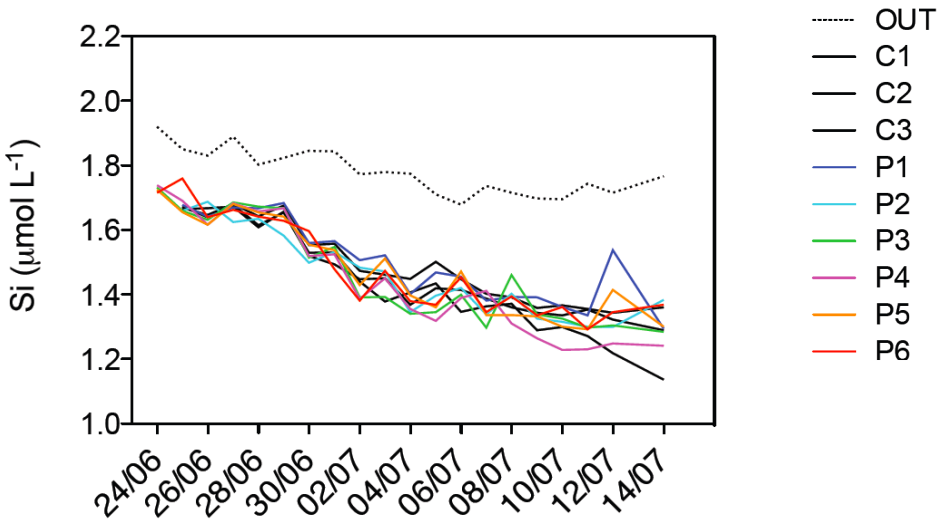

Figure 5. Integrated silicate concentrations during the course of the experiment in all nine mesocosms and in the bay of Calvi.

The oxygen saturation (expressed as percentage of saturation) is shown in Figure 3.b. It was relatively stable in the first half of the experiment (until 30th June) in all mesocosms with values below to 105 % of saturation. From the 1st July the oxygen saturation decreased gradually reaching values below to 100 % of saturation in all mesocosms. Similar conditions were observed to the outside environment. CTD-oxygen saturation profiles in the water column (data not shown) revealed that this parameter was perfectly vertically homogeneous during the whole experimental period.

3.4. PARTICULATE ORGANIC CARBON

POC values did not show a clear trend, ranging between ~3.5 µmol L^{-1} and ~4.5 µmol L^{-1} (Figure 6), with lower values at the beginning of the experiment and higher values on June 29th and 30th. POC concentrations outside the mesocosms (OUT) were generally higher than inside the mesocosms, ranging between ~5 µmol L^{-1} and ~8 µmol L^{-1}. The deviations of POC concentrations from the mean showed a not statistically significant relationship with pCO_2 (F-test, $P > 0.05$).

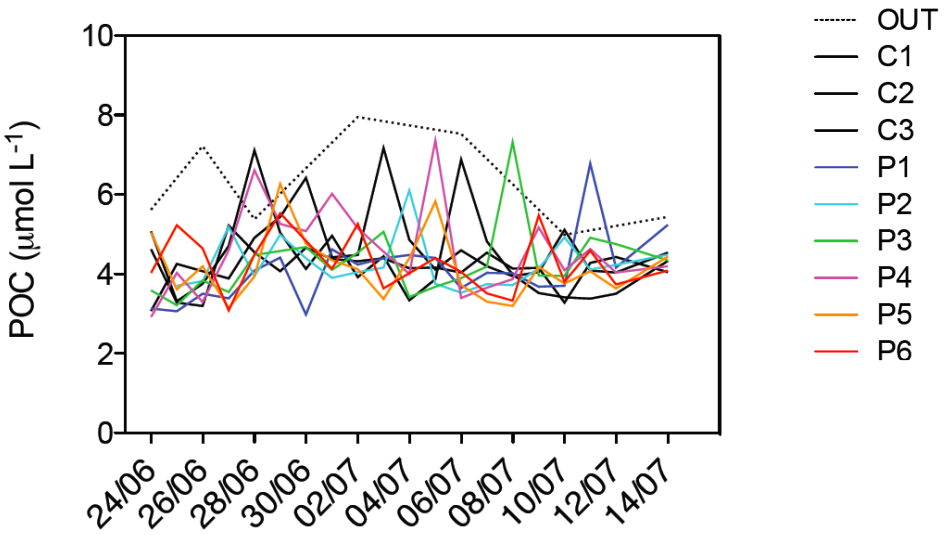

Figure 6. Integrated particulate organic carbon (POC) concentrations during the course of the experiment in all nine mesocosms and in the bay of Calvi.

3.5. COMMUNITY METABOLISM

3.5.1. NCP, CR AND GPP RATES

NCP rates in all nine mesocosms are shown on Figure 7 and were all the time relatively close to 0, ranging between -2.7 ± 0.3 µmol O_2 L^{-1} d^{-1} and 2.9 ± 0.4 µmol O_2 L^{-1} d^{-1}.

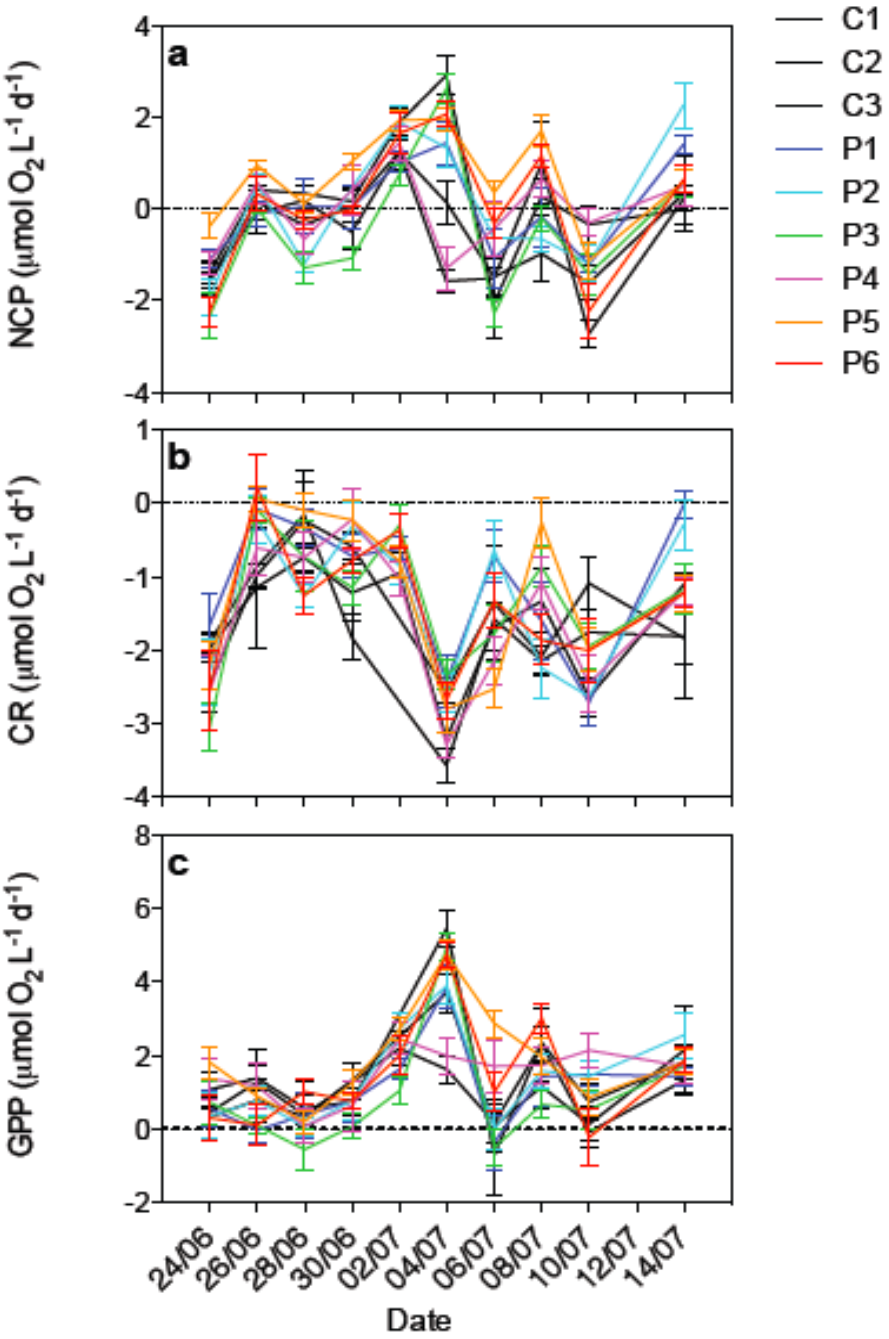

Figure 7. Metabolic rates during the whole experiment: (a) net community production (NCP), (b) community respiration (CR), and (c) gross primary production (GPP). All rates are expressed in µmol O_2 L^{-1} d^{-1} (± SE).

At the start of the experiment, the water column was heterotrophic (NCP < 0) in all mesocosms. NCP rates generally increased until July 4th when almost all mesocosms were net autotrophic at the exception of P4 and C3, and then oscillated around the metabolic equilibrium (NCP = 0), showing no real trend. CR rates varied between -3.6 ± 0.2 µmol O_2 L^{-1} d^{-1} and +0.2 ± 0.4 µmol O_2 L^{-1} d^{-1}. On several occasions, CR rates were not significantly different from 0. CR was quite variable during the experiment with a first phase showing very low rates (~ 0 to ~ -1 µmol O_2 L^{-1} d^{-1}), a peak for all mesocosms on July 4th and variations at intermediate levels until the end of the experiment, except for P1 and P2 which exhibited very low CR rates on the last incubation day. GPP varied between -0.7 ± 1.1 µmol O_2 L^{-1} d^{-1} and 5.5 ± 0.5 µmol O_2 L^{-1} d^{-1}. On seven occasions, negative rates have been calculated, however these rates were not significantly different from 0. GPP globally increased during the first half of the experiment to reach a peak in all mesocosms, except for P4 and C3. GPP oscillated thereafter between 1 and 3 µmol O_2 L^{-1} d^{-1} until the end of the experiment.

3.5.2. CUMULATIVE RATES

Cumulative rates for all mesocosms are shown in Figure 8. The cumulative NCP was negative for C2 (- 8.0 µmol O_2 L^{-1}), C3 (- 9.6 µmol O_2 L^{-1}) and P3 (- 8.25 µmol O_2 L^{-1}). The highest cumulative NCP was measured for P5 (15.42 µmol O_2 L^{-1}). Cumulative CR ranged between -40.3 µmol O_2 $L^{-1}$and -21 µmol O_2 L^{-1}. The highest cumulative GPP was measured in P5 (42 µmol O_2 L^{-1}) and the lowest in P3 (20.9 µmol O_2 L^{-1}). For all these processes, no significant relationship could be observed with pCO_2 (F-test, P > 0.05).

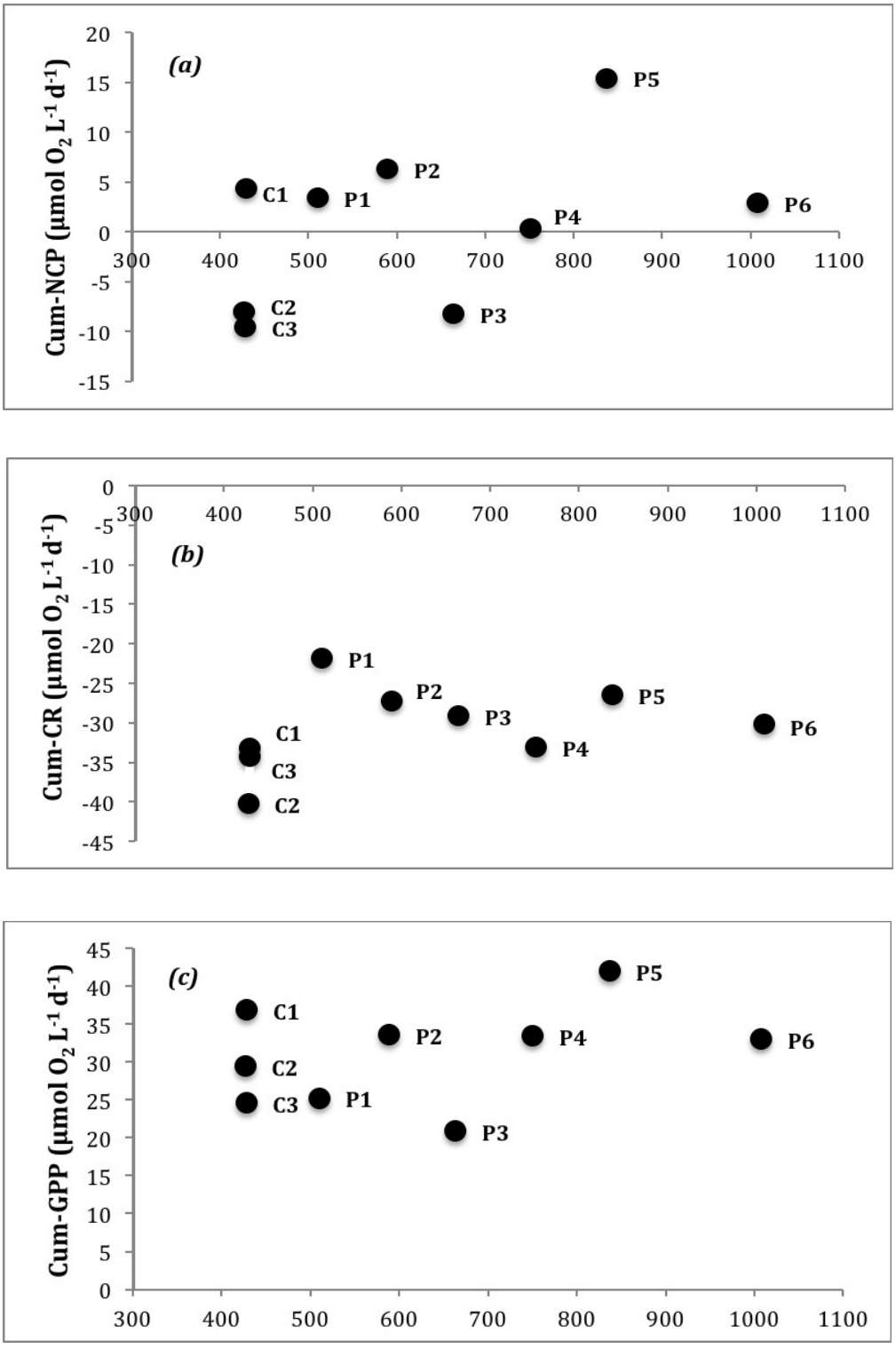

Figure 8. Cumulative values of all metabolic process during the experimentak period: (a) net community production (NCP), (b) community respiration (CR), and (c) gross primary production (GPP).

4. DISCUSSIONS

Dissolved CO_2, rather than the much more abundant bicarbonate ion, is the substrate used during the "carbon fixation" step of photosynthesis, perfomed through the enzymatic activity of the RubisCO (ribulose-1, 5-bisphosphate carboxylase oxygenase). This enzyme has low affinity for CO_2, though is higher than O_2, achieving half saturation of carbon fixation at CO_2 concentrations above those present in seawater (Badger *et al.,* 1998). Because CO_2 diffuses readily through biological membranes and leaks out of the cell it is expected that an increase in the CO_2 concentration of surface seawater will reduce CO_2 leakage, lowering the cost of concentrating CO_2 (normally against a concentration gradient). This can facilitate photosynthesis, leading to an increase in primary production. Stimulating effects of elevated CO_2 on photosynthesis and carbon fixation have indeed been observed in a variety of phytoplankton taxa, such as diatoms (Burkhardt *et al.*, 1999; Gervais & Riebesell, 2001; Wu *et al.*, 2010), coccolithophores (Barcelos e Ramos *et al*., 2010; De Bodt *et al*., 2010; Muller *et al.,* 2010; Rickaby *et al.*, 2010), cyanobacteria (Levitan *et al.,* 2007; Fu *et al.,* 2008; Kranz *et al.,* 2009) and dinoflagellates (Burkhardt *et al*., 2009; Rost *et al.,* 2006).

The extent to which phytoplankton may respond to increased CO_2 / decreased pH depends also on the physiological mechanisms of inorganic carbon uptake and intracellular assimilation, such as cellular CO_2-concentrating mechanisms (CCMs; Rost *et al.,* 2003; Giordano *et al.,* 2005). Species with effective CCMs are less sensitive to increased CO_2 levels than those with less efficient CCMs (Burkhardt *et al.,* 2001; Rost *et al.,* 2003). Because of energetic costs of CCMs, light and nutrients may affect how CO_2 regulates photosynthesis in marine phytoplankton and, as a result, the species composition, timing, and duration of phytoplankton blooms. The interactions of CO_2, nutrients, and light may also affect the geochemical cycles of elements in the sea because the C, N, and P contents and PIC:POC ratios of biogenic particles are modulated by CO_2-driven changes in cell physiology and phytoplankton species composition (Reinfelder, 2011).

Ocean acidification has direct or indirect effects both on autotrophic and heterotrophic organisms, influencing their processes, such as respectively primary

and secondary production. Changes in pCO_2 can lead to significant changes in the community structure of free-living bacteria, which were tightly correlated to phytoplankton dynamics (Allgaier *et al.*, 2008). During the Bergen mesocosm experiments (PeECE project), the apparent increase in DOC production did not stimulate bacterial secondary production due to N and P limitation as reported by Tanaka *et al.* (2008). As a matter of fact, TEP (transparent exopolymeric particles) production, DOC exudation and ensuing enhanced sedimentation must be high to stimulate secondary production. The steady increase in dissolved organic material (DOC, DON, DOP) was not significantly different between mesocosms with different pCO_2 values (Schulz *et al.,* 2008). The free-living and attached bacteria activities were negatively correlated. This condition explains that increasing concentrations of DOC throughout the bloom do not necessarily stimulate bacteria secondary production. As indicated by Tanaka and collaborators, after the peak of the phytoplankton bloom the released labile DOC may have been rich in carbon such as glucose whereas P and N were depleted.

This suggests that there is a slight but rather indirect effect of changes in pCO_2 on bacterial activities and community structure that is mainly related to phytoplankton carbon consumption, DOC exudation, as well as TEP formation and subsequent sedimentation. During the Svalbard experiment (EPOCA project), DOC production was significantly higher in CO_2 enriched mesocosms suggesting that CO_2 had a direct influence on DOC production. DOC concentrations inside the mesocosms increased more in high-CO_2 mesocosms (Engel *et al*., 2012), stimulating the activities of heterotrophic microorganism.

In contrast, in another mesocosm experiment, no significant effects of ocean acidification were found on the concentrations of chromophoric DOM (cDOM) and DOC (Engel *et al*., 2004; Grossart *et al.*, 2006; Schulz *et al.,* 2008). In on-board experiments under increased pCO_2, the production of TEP increased as a function of CO_2 uptake (Engel, 2002). In a mesocosm experiment, TEP production was significantly higher in the high pCO_2 levels (~710 μatm) than in lower pCO_2 treatments (Engel *et al.,* 2004). This indicates a possible direct effect of pCO_2 on polysaccharide exudation. The increasing DIC levels should not increase the particulate organic carbon (POC) concentrations, because of an increased TEP production, which stimulated particle aggregation and accelerated sedimentation as previously observed by Logan *et al.* (1995) and Engel (2000).

An increased TEP aggregation often results in higher abundances of

bacterial production by attracting these cells to the microbial hot spots (Simon *et al.,* 2002). Therefore, increased TEP aggregation could result in higher bacterial abundance and production (Weinbauer *et al.,* 2011).

It has been recognized that heterotrophic bacteria can be strong competitors of phytoplankton for inorganic nutrients (Thingstad & Rassoulzadegan, 1995), and bacterial processes appear to be P-limited in the oligotrophic waters of the Mediterranean Sea. Highly significant positive relationships have been shown between primary and bacterial productions in both western and eastern basins of Mediterranean Sea, indicating that primary production is a source of DOC for bacterial production in both areas, although the nature of the relationships is significantly different between the two basins.

Almost all published scientific papers were elaborated on single organisms and less than 5% of the studies have been performed on real communities or ecosystems (Gattuso & Hansson, 2011). Extrapolating knowledge from individual species studies and associating it to ecosystem is difficult because of the absence of competition or trophic interactions during most of the experiments. Several experiments have been conducted on benthic systems in natural high CO_2 environments (Hall-Spencer *et al.* 2008) or through oceanographic transects along CO_2 gradients (Charalampopoulou *et al.,* 2011) although these types of studies are limited by the numerous co-varying environmental factors with pCO_2 (Riebesell *et al.*, 2012). Mesocosms appear as the only scientific tool that allow maintaining a natural community under almost natural and self-sustaining conditions and testing the effects of a perturbation on its dynamics and functionning.

In the past few years, several mesocosm studies focusing on the effects of ocean acidification have been conducted. Three have been performed in the frame of the PeECE project (Bergen, Norway, Pelagic Ecosystem CO_2 Enrichment studies, 2001 - 2005). Most of the results have been published in a special issue of the Biogeosciences journal (http://www.biogeosciences.net/special_issue38.html). The results obtained during the European Project on Ocean Acidification (EPOCA) project in Svalbard (Kongsfjorden, Norway) in 2010 are currently under revision in a special issue of the Biogeosciences journal (http://www.biogeosciences-discuss.net/special_issue94.html).

In Bergen and in Svalbard, respectively Egge *et al.* (2009) and Engel *et al.* (2012) showed an increased carbon fixation (using the ^{14}C incorporation

technique) at high pCO_2 levels. During the Bergen experiment, Riebesell *et al.* (2007) showed an evident enhanced DIC drawdown at high pCO_2 due to the increased production of organic matter. During the Svalbard experiment, Silyakova *et al.* (2012) showed net autotrophy in the mesocosms under high CO_2 treatment, while mesocosms with intermediate and low CO_2 levels were net heterotrophic. In Bergen, using the same technique than in the present experiment (i.e. O_2 dynamics), Delille *et al.* (2005) showed no changes in NCP related to pCO_2 conditions. In a subsequent Bergen experiment, Egge *et al.* (2009) showed that NCP, CR and GPP were not dependant on pCO_2 levels. Recently, in Svalbard, and in contrast to the results of Silyakova *et al.* (2012), Tanaka *et al.* (2012) showed insignificant responses of NCP with increasing pCO_2 during the whole experimental period.

In the present experiment, no effect of increasing pCO_2 was observed on any of the measured metabolic rates. CR was relatively stable throughout the experiment in all mesocosms, and NCP was most of the time close to zero. As a result, the cumulative GPP did not vary with increasing pCO_2 level during the experiment. Furthermore, POC concentrations were not affected by acidification during the total period of the experiment. Unfortunately, DOC data were not available yet. These results suggest that elevated pCO_2 does not influenced NCP and the amount of POC produced by the summer planktonic community in the Mediterranean Sea.

The absence of any effects of high pCO_2 / low pH in our study could be explained by the environmental settings (chemical and biological). Indeed, chlorophyll and nutrients are necessary to sustain photosynthesis. Phytoplankton must acquire, from surface seawater, inorganic carbon and nutrients, including nitrogen, phosphorus and trace metals, such as iron for the formation of organic matter using energy from sunlight. Nitrogen and phosphorus are used to make proteins, nucleic acids and other fundamental compounds. Diatoms need also silicates in order to produce their tests. In certain circumstances, these nutrients can be limiting factor of the growth and production of organic matter. The consumption of silicates during the experiment suggests that phytoplankton was slowly growing, albeit with no accumulation of organic matter. The water used to fill the mesocosms had very low concentrations of NO_3, PO_4, and chlorophyll, suggesting very oligotrophic conditions. This experiment was the first one to be conducted in these types of environment. The starting concentrations of nutrients

and Chl_a during the present experiment are summarized in Table 2 and compared to those during the previous mesocosm experiments. Note that in the PeECE and EPOCA studies, the mesocosms were even further enriched with nutrients to produce artificial phytoplankton blooms.

Table 2. Summary of nutrients and Chl a concentrations at the starting conditions in 3 different mesocosm experiments (Riebesell *et al.*, 2008, for PeECE; Schulz *et al*., 2012, for EPOCA; present work, for MedSeA).

	Bergen (PeECE)	Svalbard (EPOCA)	Calvi (MedSeA)
Chl_a (µg L^{-1})	2	~ 0.5	< 0.10
Si (µmol L^{-1})	> 3	> 0.5	< 2
PO_4 (µmol L^{-1})	~ 0.7	> 1	< 0.07
NO_3 (µmol L^{-1})	~ 15	~ 0	< 0.1
NH_4^+ (µmol L^{-1})	NA	> 0.5	< 0.5

Another explanation would be that phytoplanktonic species present in the mesocosms had very efficient CCMs, which may have limited the theoretical stimulating effects of pCO_2 on photosynthesis. Unfortunately, at the time of finalizing this thesis, results on the diversity of the planktonic community were not available.

It has to be stressed that ocean acidification will not the only perturbation potentially impacting the ocean The Mediterranean Sea is a largely enclosed sea, with high temperature and salinity, showing consistent surface warming rates of 0.026 - 0.033 °C $year^{-1}$ driven by regional phenomena (Bensoussan *et al.,* 2009).

On the Catalan coast (Spain), Coma *et al*. (2009) showed enhanced stratification with a ca. 40% higher since 1974. This increase in temperature can lead to impacts on the Mediterranean Sea role in the global carbon cycle, through changes in solubility (CO_2 solubility decreases with increasing temperature) and biological carbon pumps. Indeed, most of nutrient supply to the surface ocean comes from the mixing and upwelling of cold, nutrient - rich water from below. Warming of surface waters increases stratification, inhibits mixing and reduces the upward nutrient supply and therefore decreases productivity (Doney, 2006).

The effect of global change on photosynthesis has focused on the importance of thermal stratification for light supply and nutrient availability with any effects from direct temperature influence. Indeed, temperature increase provokes an acceleration of physiological rates and the organic matter production

of light-limited phytoplankton appears to be less responsive to temperature than heterotrophic processes. This parameter is suspected to affect the magnitude, timing and composition of phytoplanktonic blooms. However, considering the oligotrophic nature of Mediterranean Sea, seawater warming, as we have seen for acidification, will most likely not have significant effects on photosynthetic rates, and we expect that the increase of the water column stratification will even increase these nutrient-limiting conditions and decrease primary production rates, as suggested by Behrenfeld *et al.* (2006) for the world ocean. Nevertheless, it seems clear that seawater warming will induce a shift in dominant species toward small-sized cells, an effect that has been observed also in several ocean acidification studies (Legendre & Rivkin, 2002; Riebesell *et al.,* 2009). All in all, this will certainly have impacts on the capacity of the surface water ecosystems to provide organic matter for higher trophic levels and the deep-sea, as well as its capacity to pump atmospheric CO_2, therefore minimizing the role of the ocean as a sink for anthropogenic CO_2.

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