No detectable effect of ocean acidification on plankton metabolism in the NW oligotrophic Mediterranean Sea: results from two mesocosm studies

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Authors: L. Maugendre[1,2*], J.-P. Gattuso[1,2], A. J. Poulton[3], W. Dellisanti[1,2], M. Gaubert[1,2], C. Guieu[1,2] and F. Gazeau[1,2]

[1] Sorbonne Universités, UPMC Univ Paris 06, UMR 7093, LOV, Observatoire océanologique, F-06230, Villefranche-sur-mer, France

[2] CNRS, UMR 7093, LOV, Observatoire océanologique, F-06230, Villefranche-sur-mer, France

[3] National Oceanography Centre, University of Southampton Waterfront Campus, European Way, United Kingdom

*Corresponding author: laure.maugendre@gmail.com

Highlights:

- Two sites with different metabolic status
- Summer conditions close to metabolic balance in the Bay of Calvi
- Winter autotrophic conditions in the Bay of Villefranche, with no bloom
- No effect of ocean acidification on plankton metabolic rates at both sites
- Natural environmental limitations override a potential effect of ocean acidification

Keywords: ocean acidification, plankton community, metabolic rates, mesocosm experiments, Mediterranean Sea

Abstract

Oligotrophic areas account for about 30% of oceanic primary production and are projected to expand in a warm, high-CO_2 world. Changes in primary production in these areas could have important impacts on future global carbon cycling. To assess the response of primary production and respiration of plankton communities to increasing partial pressure of CO_2 (pCO_2) levels in Low Nutrient Low Chorophyll areas, two mesocosm experiments were conducted in the Bay of Calvi (Corsica, France) and in the Bay of Villefranche (France) in June-July 2012 and February-March 2013 under different trophic state, temperature and irradiance conditions. Nine mesocosms of 50 m^3 were deployed for 20 and 12 days, respectively, and were subjected to seven pCO_2 levels (3 control and 6 elevated levels). The metabolism of the community was studied using several methods based on *in situ* incubations (oxygen light-dark, ^{18}O and ^{14}C uptake). Increasing pCO_2 had no significant effect on gross primary production, net community production, particulate and dissolved carbon production, as well as on community respiration. These two mesocosm experiments, the first performed under maintained low nutrient and low chlorophyll, suggest that in large areas of the ocean, increasing pCO_2 levels may not lead to a significant change in plankton metabolic rates and sea surface biological carbon fixation.

1. Introduction

Oceanic primary production represents about 50% of global primary production (Field et al., 1998) and plays a key role in climate regulation. The balance between gross primary production (GPP) of autotrophic organisms and community respiration (CR) of both autotrophic and heterotrophic organisms determines the net community production (NCP), revealing the capacity of a system to sequester carbon via the biological pump. Production and consumption of organic matter depend on the composition of the plankton community and are constrained by environmental parameters such as nutrient availability (i.e., nitrogen, phosphorus, silicon concentration, ratios and chemical forms), light availability and temperature. The increase in the partial pressure of CO_2 (pCO_2) in the ocean and the consequent decrease in seawater pH, so-called ocean acidification (Gattuso and Hansson, 2011), might also influence the metabolism of plankton organisms and marine communities.

Many laboratory studies, focused on phytoplankton strains maintained in culture, have been performed to test the response of primary production to increased pCO_2, but present two major downsides. First of all, they do not take into account any potential compensation between species. Although laboratory studies have shown that diatoms appear to generally benefit from an increase in CO_2 and that the response of coccolithophores is more variable (from increased production to neutral or even inhibitory effects under nitrogen limitation; see comprehensive review by Riebesell and Tortell, 2011), the global response of the community might not be the sum of these individual effects. Another drawback of single strain culture experiments is that the heterotrophic component of plankton communities is, for the most part, not taken into consideration. Yet, a possible indirect effect of elevated pCO_2 on bacteria has been suggested and linked to changes in phytoplankton activity (Grossart et al., 2006). Autotrophic organisms can indeed release dissolved organic carbon (DOC), which can in turn be used by bacteria for growth and respiration. An increase in DOC production under elevated pCO_2 could therefore have an impact on the bacterial community (see also Liu et al., 2010).

In order to measure plankton metabolic rates, several techniques are available although each of these methods presents some advantages and disadvantages. The radioactive ^{14}C incorporation technique (Steemann-Nielsen 1952) has been widely used for many years. However, although this method is believed to provide accurate estimates of carbon incorporation rates (Williams et al. 1983), uncertainties still remain on what is actually measured, considered to be in between gross and net production (Peterson 1980). The oxygen light-dark method (e.g., Riley 1939) is also an accurate technique that has been used for a long time and that allows determining the metabolic state of the community (NCP). However, in order to estimate GPP rates, one has to assume that light respiration equals dark respiration (measured; CR), an assumption that is not always correct (e.g., Bender et al. 1987). In contrast, another method based on the heavy isotope of oxygen (^{18}O; Grande et al. 1982) provides very accurate and direct estimates of GPP. However, with this method all the O_2 produced is labelled even though not all this O_2 is directly linked to carbon assimilation, therefore GPP-^{18}O is believed to overestimate true GPP (Laws et al., 2000). Finally, all three methods present the disadvantage to be performed in closed small containers that might lead to some confinement effects and not completely reflect *in situ* conditions of light, nutrients, turbidity, etc. This is out of the scope of this paper to extensively discuss how these methods compare to each other; we therefore refer to detailed reviews and comparison analyses for further details (e.g. Bender et al. 1987; Gazeau et al. 2007; Regaudie-de-Gioux et al. 2014).

Experiments have recently been conducted to assess the effects of ocean acidification on natural plankton assemblages with results showing either increased photosynthesis and/or net community production with increasing pCO_2 (e.g., Riebesell et al., 2007; Egge et al., 2009) or no effect (e.g., Hare et al., 2007; Tanaka et al., 2013). Some of these experiments at the community level have been conducted using pelagic mesocosms. This approach is considered to be closer to the “real world” because large mesocosms enclose a significant volume of

seawater containing an entire plankton community with environmental conditions (e.g., temperature, irradiance, water motion) within the mesocosm similar to those prevailing *in situ* (Riebesell et al., 2010, 2013). However, most of these experiments have been performed in high-nutrient or nutrient-enriched systems and very few experiments have been reported in low nutrient areas (Yoshimura et al., 2010). Yet, pelagic primary production is highly variable between oceanic provinces and more than 60% of the open ocean is considered to be oligotrophic (i.e. low chlorophyll). Despite their low nutrient concentration and relatively low productivity, these areas represent about 30% of oceanic primary production (Longhurst et al., 1995). Furthermore, it has been suggested that oligotrophic areas will expand as a result of ocean warming (Polovina et al., 2008), with potential implications for ocean biogeochemistry and primary production (Irwin and Oliver, 2009). Although the metabolic status of open ocean waters is still hotly debated (Duarte et al., 2013; Williams et al., 2013), any change due to ocean acidification and/or warming will undoubtedly have profound impacts on the biological carbon pump and carbon cycle. Most of the oligotrophic areas are in the open ocean where it is difficult to perform field experiments. The Mediterranean Sea, a semi-enclosed sea, gives the opportunity to overcome this problem as characterized by low nutrient and low chlorophyll (LNLC) concentrations, although depending on the location and season, trophic conditions can be defined as ranging from mesotrophic to ultra-oligotrophic (D'Ortenzio and d'Alcalà, 2009).

To test whether ocean acidification will affect plankton community composition and functioning in oligotrophic areas, two mesocosm experiments were performed in the North-Western Mediterranean Sea during two contrasting periods (winter *vs.* summer), in the framework of the European project 'Mediterranean Sea Acidification in a Changing Climate' (MedSeA; www.medsea-project.eu). Here, we report on the effects of ocean acidification on plankton metabolism (gross primary production, net community production, particulate and

dissolved carbon production as well as community respiration), as measured using the methods briefly described above (the oxygen light-dark, ^{14}C and ^{18}O labelling techniques).

2. Material and Method

2.1. Study sites and experimental set-up

One mesocosm experiment was conducted in the Bay of Calvi (BC; Corsica, France) in summer (June-July 2012) and the other one in the Bay of Villefranche (BV; France) during the transition between winter and spring (February-March 2013). The experimental set-up and mesocosm characteristics are fully described in a companion paper (Gazeau et al., sbm, this issue). Briefly, for each experiment, nine mesocosms of ca. 50 m^3 (2.3 m in diameter and 12 m deep) were deployed for 20 and 12 days in BC and BV, respectively. Once the bottom of the mesocosms was closed, CO_2 saturated seawater was added to obtain a pCO_2 gradient across mesocosms ranging from ambient levels to 1,250 µatm (Table 1), with three control mesocosms (C1, C2 and C3) and six mesocosms with increasing pCO_2 (P1 to P6). Measurements of plankton metabolism started after the end of the CO_2 manipulation, on 24 June 2012 and 22 February 2013 for BC and BV, respectively corresponding to Day 0 in BC and Day 1 in BV. Before sunrise (04:00 in BC and 05:00 in BV; local times), depth-integrated sampling (0 to 10 m) was performed using 5 L Hydro-Bios integrated water samplers and distributed into various incubation bottles (see below). Processes influenced by light were incubated *in situ* on an incubation line, moored near the mesocosms, and incubations took place at the depth of mean irradiance over the 12 m depth of the mesocosms (6 m for BC and 4 m for BV; see section on irradiance below for more details). Other incubations were performed in a laboratory incubator at *in situ* temperature (ca. 21-25 °C for BC and ca. 13 °C for BV). During both experiments, net community production (NCP) and community respiration (CR) were determined using the oxygen light-dark method every two days. Gross primary production (GPP) was measured using the ^{18}O-labelling method (GPP-^{18}O) every 4 days during the BC experiment, while rates of particulate organic (PP-^{14}C) and dissolved organic carbon production (DO^{14}Cp) were determined every two days using the ^{14}C labelling

technique during the BV experiment.

2.2. Irradiance and metabolic rates measurements techniques

2.2.1. Irradiance

Surface irradiance (photosynthetically active radiation; PAR) was measured continuously during the two experiments using a LI-COR LI-192SA 2-Pi sensor connected to a LI-1400 data logger (see Gazeau et al., sbm, this issue, for more details). The depth of mean irradiance was estimated at the start of each mesocosm experiment based on PAR profiles (0 to 12 m) performed using a Biospherical Instruments Inc. QSP-2200 4-Pi sensor mounted on a CTD SBE 19plusV2. Thereafter, PAR profiles (0 to 12 m) were conducted daily at the incubation sites to estimate vertical attenuation coefficients ($K_{d[PAR]}$). For each incubation day, the mean daily irradiance at the incubation depth was calculated using surface PAR and the attenuation coefficient.

2.2.2. Oxygen light-dark method

From each mesocosm, 15 biological oxygen demand (BOD; 60 mL) bottles were filled, among which five were immediately fixed with Winkler reagents and used to estimate initial dissolved oxygen (O_2) concentrations. Five transparent bottles were incubated *in situ* on the incubation line for 24 h to estimate NCP (sunrise to sunrise). In order to estimate CR, 5 bottles were incubated for 24 to 36 h in the dark in a laboratory incubator at *in situ* temperature (ca. 21-25 °C for BC and ca. 13 °C for BV). Upon completion of the incubations, samples were fixed with Winkler reagents. O_2 concentrations were measured using an automated Winkler titration technique with potentiometric end-point detection. Analyses were performed with a Metrohm Titrando 888 and a redox electrode (Metrohm Ag electrode). Reagents and standardizations were similar to those described by Knap et al. (1996). NCP and CR were estimated by regressing O_2 values against time, and CR were expressed as negative values. Gross primary production (GPP-O_2) rates were calculated as the difference between

NCP and CR. The combined errors were calculated as S.E. $_{x-y} = \sqrt{(S.E._x^2 + S.E._y^2)}$.

2.2.3. GPP-^{18}O method

In BC, every 4 days, water samples from each mesocosm were transferred into eight transparent glass bottles (60 mL) and sealed. Three bottles were immediately poisoned with 10 µL of a saturated mercury chloride ($HgCl_2$) solution in order to estimate the initial O_2 isotopic composition. The remaining five transparent glass bottles were spiked with 100 µL of 97% $H_2{}^{18}O$ in order to reach a $\delta^{18}O$-H_2O enrichment of 650‰ and were incubated *in situ* from sunrise to sunset. Upon completion of the incubation, samples were poisoned using 10 µL of $HgCl_2$, and stored upside down in the dark at room temperature pending analysis. Isotopic measurements were performed at Leuven University (Belgium). A headspace of 3 mL was created with helium and allowed to equilibrate for 30 min in order to measure ^{18}O-O_2. The extracted water was then injected into helium-flushed vials for ^{18}O-H_2O measurements. Pure CO_2 (100 µL) was then added and samples were allowed to equilibrate for 24 h. $\delta^{18}O$-H_2O was therefore measured as $\delta^{18}O$-CO_2. Determinations of $\delta^{18}O$-O_2 and $\delta^{18}O$-CO_2 were performed on an elemental analyzer (Flash HT/EA) coupled to a Delta V Isotope-ratio Mass Spectrometer (IRMS). An overflow technique was used to limit air contamination of the needle. For $\delta^{18}O$-O_2, the internal standard used to correct the data and monitor instrumental drift was air from the outside. For $\delta^{18}O$-CO_2, a calibration was performed against Vienna Standard Mean Ocean Water (VSMOW). GPP-^{18}O rates (µmol O_2 L^{-1} d^{-1}) were calculated using the following equation (Kiddon et al., 1995):

$$GPP\text{-}^{18}O = [(\delta^{18}O\text{–}O_{2final} - \delta^{18}O\text{–}O_{2init}) / (\delta^{18}O\text{-}H_2O - \delta^{18}O\text{–}O_{2init})] \times O_{2init}$$

where $\delta^{18}O$-O_{2init} and $\delta^{18}O$-O_{2final} are measured $\delta^{18}O$-O_2 before and after incubation (‰), $\delta^{18}O$-H_2O is the final isotopic composition of the labelled seawater (‰), and O_{2init} is the O_2 concentration before incubation (µmol O_2 L^{-1}). The overall error was estimated using a Monte-Carlo procedure where one thousand values were randomly chosen between the mean

± S.D. of each measured parameter and the mean ± S.E. of each computed parameter is reported (Pemberton et al, 2006).

2.2.4. ^{14}C primary production

In BV, every 2 days, water samples from each mesocosm were transferred to four culturing flasks (40 mL) and spiked with 0.37 to 1.85 MBq (10 to 50 µCi) of a ^{14}C-labelled sodium bicarbonate solution. Three flasks were incubated *in situ* for 24 h (sunrise to sunrise). The remaining flask was immediately poisoned with 1 mL of a borax-buffered formaldehyde solution filtered through a 0.2 µm syringe tip filter and stored in the laboratory to estimate abiotic ^{14}C labelling. After 24 h, the samples were brought back to the laboratory and 3 mL was gently filtered through 0.2 µm polycarbonate filters directly into scintillation vials for DO^{14}Cp measurements (López-Sandoval et al., 2011). Scintillation vials were closed with a gas-tight rubber stopper and plastic centre wells containing a GF/A filter soaked with 200 µL of *ß*-phenylethylamine. Then, 75 µL of hydrochloric acid (HCl; 50%) was injected into the vial in order to transform ^{14}C-DIC to $^{14}CO_2$, which was trapped by the *ß*-phenylethylamine while ^{14}C-labelled DOC remained in the seawater.

The remaining 37 mL was then filtered through 0.4 µm polycarbonate filters (25 mm diameter) and rinsed with freshly filtered (0.7 µm) seawater. Filters were placed in scintillation vials that were closed with gas-tight rubber stopper and centre wells with a GF/A filter soaked with *ß*-phenylethylamine, as for DO^{14}Cp. One mL of phosphoric acid (H_3PO_4; 1%) was injected through the rubber stopper onto the filter in order to dissolve ^{14}C-particulate inorganic carbon (Balch et al., 2000). After another 24 h, the centre wells and soaked GF/A filters were placed separately into fresh scintillation vials. Scintillation cocktail (15 mL; Ultima Gold MV, Perkin Elmer) was added to the vials containing the DOC (DO^{14}Cp) and the PC filter (PP-^{14}C) and activities were determined on a Packard Tri Carb (1600 CA) scintillation counter. Disintegrations per minute (DPM) were converted to production rates

(after correction from abiotic ^{14}C labelling) using dissolved inorganic carbon concentrations measured in the mesocosm (Gazeau et al., sbm, this issue) and an isotopic discrimination factor of 1.05. In order to verify the initial spike activity, 100 µL of seawater from 3 to 6 random culture flasks were removed and placed in a scintillation vial containing 200 µL of *ß*-phenylethylamine and these were counted on the scintillation counter. The percentage of extracellular release (PER) was calculated as $DO^{14}Cp/(PP\text{-}^{14}C + DO^{14}Cp)$ (López-Sandoval et al., 2011).

2.3. Data analysis, statistics and data availability

Results are reported as mean value ± standard error (S.E.) as well as the average over all mesocosms ± standard deviation (S.D.) when specified. Cumulative metabolic rates were calculated for the whole experimental period. Values for days when no incubations were performed were obtained by linear interpolation and the cumulative values were then summed up for the experimental period. The combined errors were calculated as $S.E._{x-y} = \sqrt{(S.E._x^2 + S.E._y^2)}$. The pCO_2 values used for the representation of cumulative metabolic rates are the average pCO_2 over the experimental period for each mesocosm. To test for pCO_2 increase effects, the relationship between cumulated metabolic rates and pCO_2 were realised using linear regressions. Linear regressions were also used to test for relationships between production rates and time or PAR, while Model-II linear correlation were used to compare metabolic rates obtained with the different measurements methods. All linear regression and correlation were performed using the R software (R Core Team, 2013) and were considered significant at a probability $p < 0.05$. The data sets are freely available on Pangaea, in the Bay of Calvi: http://doi.pangaea.de/10.1594/PANGAEA.810331 and in the Bay of Villefranche: http://doi.pangaea.de/10.1594/PANGAEA.835117.

3. Results

3.1. Summer conditions (Bay of Calvi)

The initial and mean pCO_2 values over the experiment are presented in Table 1 while initial temperature, salinity, and concentrations of nutrients and chlorophyll *a* inside and outside the mesocosms in the Bay of Calvi (BC) are shown in Table 2. Further details regarding temperature and salinity can be found in Gazeau et al. (sbm, this issue) and analytical protocols used to measure nutrients and chlorophyll *a* concentrations are available in Louis et al. (in prep, this issue) and Gazeau et al. (in prep, this issue), respectively. At the start of the experiment (day 0), the concentration of nitrogen (NO_x = nitrate + nitrite) was similar inside and outside the mesocosms. In contrast, the concentrations of dissolved inorganic phosphate (DIP) and chlorophyll *a* were lower inside than outside the mesocosms (Table 2).

NCP ranged from -2.7 ± 0.3 to 2.9 ± 0.4 µmol O_2 L^{-1} d^{-1} over the experimental period of 20 days (Figure 1 a). The lowest and highest values were measured in the control mesocosms, respectively C3 on day 16 and C1 on day 10. NCP was negative on day 0 and tended to increase and reached a maximum value on day 8 or 10 (day 20 for P2), depending on the mesocosm. After this period of increase, NCP remained close to metabolic balance (ca. 0 µmol O_2 L^{-1} d^{-1}) until the end of the experiment. CR varied from -3.6 ± 0.2 to 0.2 ± 0.4 µmol O_2 L^{-1} d^{-1} (Figure 1 b). The lowest and highest values were measured in C2 on day 10 and P6 on day 2, respectively. Similar to NCP, the highest CR (i.e. the most negative) were measured on day 10 in all mesocosms apart from P2 for which it was measured on day 16 (-2.63 ± 0.20 µmol O_2 L^{-1} d^{-1}).

GPP-O_2 ranged from -0.7 ± 1.1 to 5.5 ± 0.5 µmol O_2 L^{-1} d^{-1} (Figure 1 c). The lowest and highest rates were both measured in C1, on day 12 and day 10, respectively. After a stable period from day 0 to day 6, GPP-O_2 increased to reach a maximum value on day 10 for all

mesocosms except C3 and P4, for which maximum values were reached on day 14 and day 8, respectively. GPP-^{18}O varied from 0.0 ± 0.1 to 1.7 ± 0.1 µmol O_2 L^{-1} d^{-1} (Figure 2). The lowest value was measured in C2 on day 0, while the highest value was measured in P4 on day 16. GPP-^{18}O was relatively stable during the experiment, showing a slight increase until day 16 and a decrease on day 20 (except for C2, which decreased from day 12). GPP-^{18}O rates were generally lower than GPP-O_2, with no significant relationship ($r^2 < 0.01$, $p > 0.05$, n = 52).

Mean daily PAR at 6 m (Figure 3) was constant through time (r = 0.04, $p > 0.05$, n = 10) varying from 180 and 330 µmol photon m^{-2} s^{-1}. GPP-O_2, GPP-^{18}O and NCP were significantly related to the PAR at 6 m (GPP-O_2: r = 0.54, $p < 0.05$, n = 88; GPP-^{18}O: r = 0.27, $p < 0.05$, n = 54; NCP: r = 0.30, $p < 0.05$, n = 90; respectively).

Cumulative NCP, averaged over all nine mesocosms (± S.D.), was -1 ± 8 µmol O_2 L^{-1} and varied between -11.9 ± 1.8 and 13.6 ± 1.2 µmol O_2 L^{-1} (Figure 4 a) depending on the mesocosm with the lowest rates measured in C2, C3 and P3 and the highest estimated in P5. There was no significant trend in cumulative NCP with increasing pCO_2 (r = 0.44, $p > 0.05$, n = 9). The average (± S.D.) cumulative CR was -29 ± 4 µmol O_2 L^{-1}, with no significant trend with increasing pCO_2 (r = 0.30, $p > 0.05$, n = 9). The cumulative GPP-O_2 and GPP-^{18}O were on average (± S.D.) 28 ± 6 µmol L^{-1} and 20 ± 4 µmol O_2 L^{-1}, respectively. For both methods there was no significant trend with increasing pCO_2 (r = 0.47, $p > 0.05$, n = 9 and r = 0.17, $p > 0.05$, n = 9, respectively).

Normalizing time course and cumulative metabolic rates to chlorophyll *a* concentrations (Gazeau et al., in prep, this issue) led to similar results regarding the effect of pCO_2, the relationships between methods and with PAR (data not shown).

3.2. Winter-spring conditions (Bay of Villefranche)

The initial and mean pCO_2 values over the experiment are presented in Table 1 while

initial temperature, salinity, and concentrations of nutrients and chlorophyll *a* inside and outside the mesocosms in BV are shown in Table 2. Further experimental details can be found in Gazeau et al. (sbm, this issue). When sampling started (day 0), NO_x concentrations were higher outside than inside the mesocosms (Table 2) and remained higher outside the mesocosm during the experimental period (Louis et al., in prep, this issue). In contrast, DIP and chlorophyll *a* concentrations inside and outside the mesocosms were initially similar (Table 2).

NCP varied from -2.0 ± 0.4 to 2.8 ± 0.5 µmol O_2 L^{-1} d^{-1} (Figure 1 d). The lowest and highest values were both in C1, on day 1 and on day 9, respectively. NCP generally increased ($r = 0.57$, $p < 0.05$, $n = 52$) throughout the experiment from negative (heterotrophic system) to positive values (autotrophic system). NCP was negative on day 1 for all mesocosms except C3, P2 and P4 whereas, on day 5, all mesocosms had positive NCP. CR ranged from -3.7 ± 0.4 µmol O_2 L^{-1} d^{-1} in P3 on day 1 (overall mean ± S.D.: -2.6 ± 0.6 µmol O_2 L^{-1} d^{-1}) to 0.02 ± 0.47 µmol O_2 L^{-1} d^{-1} in P6 on day 9 (overall mean ± S.D.: -0.9 ± 0.6 µmol O_2 L^{-1} d^{-1}; Figure 1 e) and generally decreased with time (i.e. becoming less negative; $r = 0.63$, $p < 0.05$, $n = 54$). GPP-O_2 ranged from 0.8 ± 0.6 to 3.6 ± 0.6 µmol O_2 L^{-1} d^{-1} (Figure 1 f) with a slight increasing trend as a function of time ($r = 0.43$, $p < 0.05$, $n = 54$).

PAR at 4 m significantly increased during the experiment from 30 to 190 µmol photon m^{-2} s^{-1} (Figure 3; $r = 0.80$, $p < 0.05$, $n = 6$). GPP-O_2 and NCP increased significantly with PAR ($r = 0.40$, $p < 0.05$, $n = 54$ and $r = 0.76$, $p < 0.05$, $n = 54$, respectively).

Cumulative NCP, averaged (± S.D.) over all nine mesocosms, was 7.4 ± 2.6 µmol O_2 L^{-1} and varied from 3.7 ± 0.8 to 11.8 ± 1.6 µmol O_2 L^{-1} (Figure 4 b). Cumulative CR was on average -17.3 ± 2.8 µmol O_2 L^{-1} and, as for cumulative NCP, minima and maxima were measured in control mesocosms (C1 and C3, respectively) while the average GPP-O_2 was 24.7 ± 2.7 µmol O_2 L^{-1}. Cumulative NCP, CR and GPP-O_2 did not show any significant trend

with increasing pCO_2 (NCP: r = 0.06, $p > 0.05$, n = 9; CR: r = 0.04, $p > 0.05$, n = 9 and GPP-O_2: r = 0.02, $p > 0.05$, n = 9).

Primary production measured with the ^{14}C labelling technique did not show any temporal trend and was highly variable from one day to the next (Figure 5). PP-^{14}C were slightly lower outside than inside the mesocosms and varied from 0.35 ± 0.00 to 0.80 ± 0.04 µmol C L^{-1} d^{-1} (Figure 5). During the first part of the experiment (from day 3 to 5), DOC production rates (DO^{14}Cp) were highly variable both between days and between mesocosms. During the second part of the experiment (from day 7 to the end), this variability decreased and rates were relatively constant with an overall average (± S.D.) of 0.21 ± 0.11 µmol C L^{-1} d^{-1} (Figure 5). TO^{14}C production rates (PP-^{14}C + DO^{14}Cp) varied from 0.50 ± 0.0 to 2.6 ± 0.1 µmol C L^{-1} d^{-1}. PER generally decreased throughout the experiment (r = -0.58, $p < 0.05$, n = 52) and averaged (± S.D.) 25 ± 12% (from 11 to 61%).

Cumulative PP-^{14}C, DO^{14}Cp and TO^{14}C averaged (± S.D.) 7.1 ± 0.8, 2.6 ± 0.6 and 9.6 ± 0.9 µmol C L^{-1} respectively and did not show any trend with increasing pCO_2 (Figure 6; PP-^{14}C: r = 0.46, $p > 0.05$, n = 9; DO^{14}Cp: r = 0.05, $p > 0.05$, n = 9 and TO^{14}C: r = 0.38, $p > 0.05$, n = 9, respectively).

Oxygen light-dark and ^{14}C primary production methods were compared except for the first experimental day as the O_2-LD method provided negative values for NCP that cannot be measured with the ^{14}C method. Using data from days 3 to 11, there was no significant relationship between NCP and TO^{14}C rates ($r^2 = 0.06$, $p > 0.05$, n = 43) but with PP-^{14}C (r^2 = 0.21, $p < 0.05$, n = 45). PP-^{14}C was closer to NCP than to GPP-O_2 (see comparable cumulative values between NCP and PP-^{14}C) with GPP-O_2 always higher than PP-^{14}C. Significant relationships were found between GPP-O_2 and TO^{14}C ($r^2 = 0.14$, $p < 0.05$, n = 43) and between GPP-O_2 and PP-^{14}C ($r^2 = 0.31$, $p < 0.05$, n = 45). An average (± S.D.) photosynthetic quotient (PQ; molar flux of gross O_2 produced/total ^{14}C consumed) of 2.02 ±

0.64 was calculated.

Normalizing time course and cumulative metabolic rates to chlorophyll *a* concentrations (Gazeau et al., in prep, this issue) led to similar results regarding the effect of pCO_2, the relationships between methods and with PAR (data not shown).

4. Discussion

4.1. Characteristics of the study sites

The mesocosms were initially filled with seawater with very low nutrient and chlorophyll concentrations (NO_x < 0.10 µmol L^{-1}; DIP < 26 nmol L^{-1}; chl *a* < 0.25 µg L^{-1}) in BC to low nutrient and chlorophyll concentrations (NO_x < 1.2 µmol L^{-1}; DIP < 20 nmol P L^{-1}; chl *a* < 1.5 µg L^{-1}) in BV. The conditions in BC were typical of the summer stratified period. The initial concentration of nutrients was higher during the pre-bloom mesotrophic conditions in BV than in BC but nutrients were rapidly consumed and concentrations were relatively low when sampling started. Both experiments were therefore characteristic of low nutrient low chlorophyll areas (LNLC).

Although the availability of nutrients and concentrations of chlorophyll *a* were higher during winter in BV, GPP rates based on the oxygen light-dark method were similar during the two experiments. This suggests that during the winter period in BV, the community was limited by nutrients as well as by light and temperature. The chlorophyll *a* data obtained at Point B in BV in 2013 revealed that no real bloom occurred that year, although chlorophyll concentrations were maximal in April (Gazeau et al., sbm, this issue). Despite the fact the GPP-O_2 was roughly identical during the two experiments, the cumulative NCP was close to 0 in BC, suggesting a metabolic balance while was above 0 in BV, suggesting autotrophy. As a consequence of different temperature and initial pCO_2 levels (430 µatm above atmospheric equilibrium in BC and 350 µatm below atmospheric equilibrium in BV) between the two periods, surface waters were a source of CO_2 to the atmosphere in BC (summer) and a sink of atmospheric CO_2 in BV (winter). The sink status of BV in winter is in agreement with times-series data (De Carlo et al., 2013). GPP-O_2 exhibited relatively large changes in BC with a maximum value measured on day 10 that could be related to high abundance of cyanobacteria *Synechococcus* spp. and autotrophic picoeukaryotes (Gazeau et

al., in prep, this issue) and to a water column stratification (Gazeau et al., sbm, this issue). In BV, NCP increased throughout the experiment while GPP-O_2 only increased slightly. The increase in NCP is related to a weaker CR, probably caused by a decrease in particulate organic matter available for the heterotrophs (Celussi et al., in prep, this issue).

Metabolic rates measured during both experiments were within the range of previously reported rates in coastal locations of the Mediterranean Sea (Navarro et al., 2004; Gazeau et al., 2005; González et al., 2008; Bonilla-Findji et al., 2010; Ridame et al., 2014) and in open waters (Regaudie-de-Gioux et al., 2009; López-Sandoval et al., 2011). More specifically, the heterotrophic conditions encountered in BC were consistent with the summer heterotrophic conditions reported in the Bay of Palma (Spain) in 2001 (Navarro et al., 2004; Gazeau et al., 2005). Very few data are available using the GPP-^{18}O method in the Mediterranean Sea, however rates measured in June-July 2012 in BC were in the range of those found in BV during the same season in 2003 by González et al. (2008) and lower than those determined in March 2012 in BV by Maugendre et al. (in press) during the four first days of their experiment, likely as a consequence of different nutrient concentrations. Furthermore, no correlation was found between GPP measured by the O_2 light-dark and the ^{18}O labelling techniques in BC. This is in agreement with previous results obtained in the Bay of Villefranche by Maugendre et al. (in press), although González et al. (2008) reported a significant correlation at the same location. However, it must be stressed that González et al. (2008) established their correlation across a much wider range of GPP. Finally, GPP rates measured with the O_2-LD in BC (global average of 1.3 µmol L^{-1} d^{-1}) are significantly higher than primary production rates obtained with the ^{13}C labelling technique in the same area in July 2008 and 2010 by Ridame et al. (2014; ~ 0.3 - 0.4 µmol C L^{-1} d^{-1}). The ^{13}C labelling technique over 24 h was shown, like the ^{14}C labelling, to provide rates much lower than those estimated with the O_2-LD technique (Hashimoto et al., 2005).

In BV, GPP-O_2 was lower than values reported by González et al. (2008) for a similar period in winter-spring. This emphasises the pre-bloom conditions and the likely limitation of metabolic processes by temperature and light. PP-^{14}C was on average 35 ± 11% of GPP-O_2 while TO^{14}C represented 48 ± 16% of GPP-O_2. This latter percentage is in the range of 40 to 80% reported by Robinson et al. (2006) for the oligotrophic Atlantic Ocean. The release of labelled DOC (DO^{14}Cp) was low but could be measured accurately, with an average S.E. of 0.002 µmol C L^{-1} d^{-1}. PER averaged 25 ± 12% which is close to the value ~ 20% reported by Marañón et al. (2005) over a wide range of primary production rates and to the value of 23.5% measured in the Almeria-Oran front (Fernández et al., 1993). However, this is slightly lower than values measured in two Mediterranean bays (41%; González et al., 2008) and in the open Mediterranean Sea in June-July 2008 (37%; López-Sandoval et al., 2011). In BV, ^{14}C primary production was closer to NCP than GPP as it is expected for 24 h incubations (Marra and Barber, 2004).

4.2. Effects of ocean acidification on community metabolism

Despite different metabolic states (balanced in Calvi and autotrophic in Villefranche) and period (summer *vs* pre-bloom), the same absence of significant response to ocean acidification was observed for all processes at the two locations. This suggests that under nutrient and other environmental limitation (e.g., light, temperature), CO_2 enrichment may not have significant effects on plankton community metabolism. It is in contrast with the hypothesis of enhanced production with increasing pCO_2 suggested by previous experiments performed at community level under nutrient replete conditions. In fact, most of the previous experiments have been conducted with high nutrient and/or nutrient addition with the exception of Yoshimura et al. (2010), Richier et al. (2014) and Maugendre et al. (in press).

The mesocosm experiment performed in an Arctic Fjord in 2010 was, in terms of experimental set-up and duration, the most similar to our experiments albeit nutrients were

added (Riebesell et al., 2013). This experiment has shown that the plankton community metabolism was sensitive to an increase in pCO_2, although conclusions diverged depending on the employed measurement method and on the period relative to nutrient addition. This is shown in Table 3 in which responses of plankton metabolism during large mesocosm experiments are summarized. Tanaka et al. (2013) found that cumulative NCP was not affected by pCO_2 over the whole experimental period, but was negatively affected after the increase in chlorophyll *a* which followed nutrient addition. Engel et al. (2013) found that primary production measured by ^{14}C uptake significantly increased with increasing pCO_2. Other experiments have been performed using smaller mesocosms (27 m^3) in the coastal North Sea (Bergen, Norway) as part of the PeECE project (Table 3). Three consecutive experiments (2001, 2003 and 2005) showed different effects on primary production. During the first experiment, no effect was found on primary production using the oxygen light-dark method in a bloom dominated by the coccolithophore *Emiliania huxleyi* (Delille et al., 2005). No effects were also found during the second experiment in 2003 (unpublished data; see in Egge et al., 2009). In contrast, an increase of cumulative PP-^{14}C was measured during a nutrient-induced diatom bloom during the third experiment (Egge et al., 2009). This enhanced production was not detected using the oxygen light-dark method and was attributed to a lack of precision in the measurements or to an absence of effect (Egge et al., 2009). The diverse responses of primary production at the same location and at similar periods of the year during the PeECE project could be attributed to differences in nutrient concentrations and stoechiometric ratios as well as irradiance levels which strongly influence the plankton community composition and metabolism. Furthermore, it is probable that phytoplankton initial community composition (Eggers et al., 2014) as well as temporal phytoplankton evolution (Table 3) had also an important influence and led to different responses to pCO_2 increase. Other mesocosms were deployed in coastal waters of Korea in 2008 (Table 3) to

assess the effects of a CO_2 increase from present to 900 µatm combined or not to a 3 °C increase in temperature. During this experiment, in which nutrient were added, a shift from particulate to dissolved organic carbon was measured at high CO_2 level (Kim et al., 2011) as well as an increase in light utilization efficiency that was not reflected on the gross community production (Kim et al., 2013). Under negligible grazing pressure (top-down control), high CO_2 level has the potential to increase growth and primary production of phytoplankton by enhancing the inorganic carbon assimilation efficiency (Kim et al., 2013) for this community dominated by diatoms and dinoflagellates in the post-bloom period (Table 3).

It must be stressed that several other experiments focused on the effects of ocean acidification on plankton community metabolism or production but in much lower volumes than studies discussed previously. For instance, in the Bay of Villefranche, no detectable effects of increasing pCO_2 level from present level to ca. 650 µatm were observed in an experiment conducted in 4 L bottles (Maugendre et al., in press). During a microcosm (9 L bottles) experiment performed in the Okhotsk Sea (Yoshimura et al., 2010), nutrient concentrations were below detection limits and chlorophyll *a* concentrations were similar to those observed in the present study (~ 0.1 - 0.3 µg chl *a* L^{-1}). Despite no direct measurements of metabolic rates in the Okhotsk Sea which limits the possibility to compare to our experiments, increasing pCO_2 levels had no effect on POC production while DOC accumulation decreased significantly (Yoshimura et al., 2010). The lack of effect on POC production is in agreement with the results reported in the present study but the decrease in DOC accumulation stands in contrast with the lack of effect on $DO^{14}Cp$ in BV. This difference could be explained by the fact that the experiment in the Okhotsk Sea was conducted after the spring bloom with different dominating species and very low ambient pCO_2 (~ 200 µatm).

Plankton communities in the ocean can be limited or co-limited by macronutrients (Low Nutrient Low Chlorophyll; LNLC) or by micronutrient such as iron (Fe) preventing phytoplankton growth even under high nutrient levels (High Nutrient Low Chlorophyll; HNLC; Moore et al. 2013). In the Gulf of Alaska, a HNLC area, four experiments under Fe-limited conditions were performed by Hopkinson et al. (2010) and only one site presented an increase in primary production at elevated pCO_2 levels. Furthermore, the CO_2 effect was much smaller than the effect of Fe addition, as also shown in several experiments in the iron-limited areas of the Bering Sea and of the North Pacific (Endo et al. 2013; Sugie et al. 2013; Yoshimura et al., 2013, 2014). These experiments conducted between 2007 and 2009 in 12 L bottles have shown that the response in terms of POC production or photosynthetic efficiency varied depending on the studied sites as well as Fe nutritional status (see Yoshimura et al. 2014 for further details). During the first cruise, while no effect of pCO_2 was found in the North Pacific, in the Bering Sea, quantitative and qualitative changes in the production of particulate and dissolved organic matter were observed with increasing pCO_2. As large cells dominated the community in the Bering Sea while in the North Pacific small eukaryotes were more abundant, Yoshimura et al. (2013) attributed these contrasting responses to differences in plankton community composition and suggested that oceanic communities dominated by small species are less sensitive to increased pCO_2. This is also supported by our results as plankton communities during our experiments were dominated by small species (i.e., cyanobacteria *Synechococcus* spp.), haptophytes, pelagophytes, cryptophytes and chlorophytes; Gazeau et al., in prep, this issue) as well as by PeECE experiments for which a pCO_2 effect on plankton metabolism was only measured in a community dominated by diatoms (Table 3). However, in the Northwest European continental shelf under low nutrient and chlorophyll *a* concentrations, Richier et al. (2014) found, based on short-term and small volume (94 h, 4 L) experiments, that high CO_2 concentrations led, in some cases, to a

significant decrease in the growth of small phytoplankton species (< 10 μm). This effect on small cells agrees with the theoretical model of Flynn et al. (2012) suggesting that small phytoplankton species are less adapted to changes in their local pH while larger cells must face larger pH variations at short time scales (day or hours). Again, our data do not support this hypothesis.

In conclusion, these perturbation experiments were carried out in typical oligo- to meso-trophic areas in two sites with different metabolic status (summer and pre-bloom periods). In both experiments, no effect of ocean acidification on community metabolism could be detected. Plankton communities were limited by nutrient availability and other environmental parameters, and therefore an increase in CO_2 concentrations had, not surprisingly, no effect on community metabolic rates. Although the present study was not performed during a phytoplankton bloom, which is very limited in time and biomass in the study areas, our results suggest that sea surface biological carbon fixation in oligotrophic areas such as the NW Mediterranean Sea will not be enhanced by CO_2 enrichment. If these results hold true for all oligotrophic areas, there would be no negative feedback of the biological pump to atmospheric CO_2 increase. However, short perturbation events stimulating metabolic rates, such as Saharan dust deposition, nutrient fertilization (for example by water column mixing, land run off) could induce a different response to ocean acidification and should be investigated in these areas in the future. Finally, ocean acidification can act synergistically with other CO_2-related perturbations such as ocean warming. Recently, several experiments have included ocean warming and acidification in different parts of the ocean showing a stronger effect of warmer conditions (Hare et al., 2007; Feng et al., 2009; Maugendre et al., in press) than ocean acidification, highlighting the importance for future studies to consider interactions with other drivers related to climate change even under nutrient depleted conditions.

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Figure1: Net community production (NCP; a and d), community respiration (CR; b and e) and gross primary production (GPP-O_2; c and f) as a function of time during the experiment in the Bay of Calvi (left) and in the Bay of Villefranche (right).

Figure 2: Gross primary production measured with the ^{18}O-H_2O labelling (GPP-^{18}O) in the Bay of Calvi.

Figure 3: Irradiance at the incubation depth in the Bay of Calvi (empty triangle) and in the Bay of Villefranche (full circles) as a function of time.

Figure 4: Cumulative rates of net community production (NCP; diamonds), community respiration (CR; circles) as well as gross primary production estimated using the oxygen light-dark (GPP-O_2; triangles) and the ^{18}O labelling (GPP-^{18}O; cross) techniques in the Bay of Calvi (a, duration: 20 days) and in the Bay of Villefranche (b, duration: 12 days). Error bars correspond to cumulated standard errors. pCO_2 is the mean value for each mesocosm during the experiment (see Gazeau et al., sbm, this issue for further details on sampling and analytical protocols).

Figure 5: Particulate primary production (PP-^{14}C; top) and dissolved organic production (DO^{14}Cp; bottom) during the experiment in the Bay of Villefranche.

Figure 6: Cumulative production rates estimated by the ^{14}C method during the experiment in the Bay of Villefranche. PP-^{14}C: particulate primary production (up triangle); DO^{14}Cp: dissolved organic carbon production (down triangle); TO^{14}C: total organic carbon production (circle). Error bars correspond to cumulated standard errors. pCO_2 is the mean value for each mesocosm during the experiment (see Gazeau et al., sbm, this issue for further details on sampling and analytical protocols).

Table 1: Initial and mean pCO_2 levels (µatm) during the experimental period in the Bay of Calvi and Villefranche (see Gazeau et al., sbm, this issue for further details on sampling and analytical protocols).

	Mesocosm	C1	C2	C3	P1	P2	P3	P4	P5	P6
Bay of Calvi	Initial pCO_2	463	455	452	595	716	774	901	1174	1327
	Mean pCO_2	429	427	429	508	586	660	747	828	990
Bay of Villefranche	Initial pCO_2	378	347	350	494	622	690	477	932	1250
	Mean pCO_2	357	356	352	456	486	544	545	719	941

Table 2: Average environmental conditions at day 0 in all mesocosms (mean ± S.D.): temperature, salinity as well as concentrations of nitrate + nitrite (NO_x), inorganic phosphate (DIP) and chlorophyll *a* (chl *a*). BC refers to the Bay of Calvi (Corsica, France) and BV to the Bay of Villefranche (France). Further details regarding temperature and salinity can be found in Gazeau et al. (sbm, this issue) and analytical protocols used to measure nutrients and chlorophyll *a* concentrations are available in Louis et al. (in prep, this issue) and Gazeau et al. (in prep, this issue), respectively.

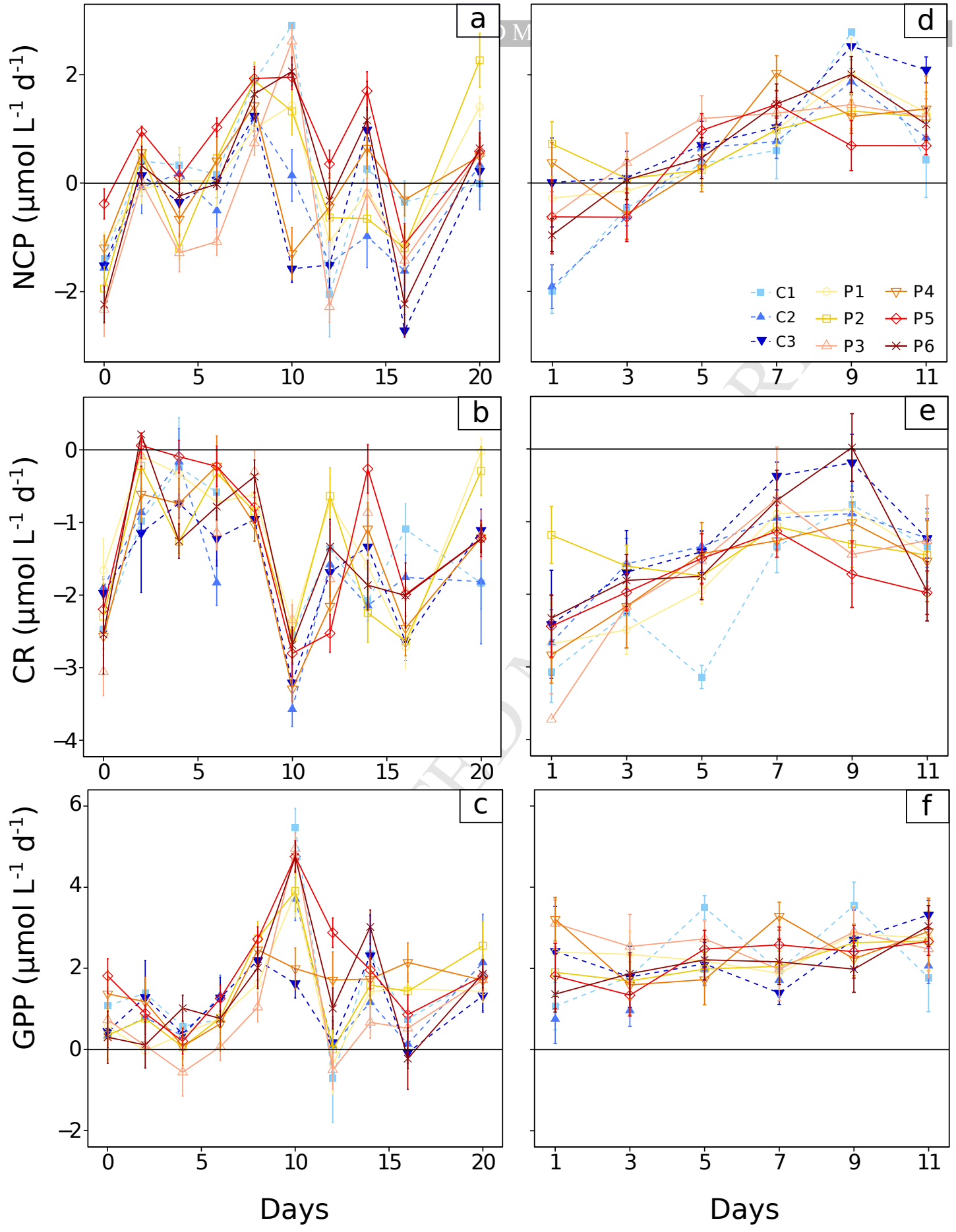
		Temperature °C	Salinity	NO_x nmol L^{-1}	DIP nmol L^{-1}	chl *a* μg L^{-1}
BC	24 June 2012					
Mesocosm	Average	22.1	37.9	47.1	22.8	0.06
	S.D.	< 0.01	< 0.01	± 14.2	± 4.1	± 0.01
Outside		22.2	38.0	49.8	34.8	0.12
BV	21 Feb 2013					
Mesocosm	Average	13.2	38.1	128.5	10.4	1.1
	S.D.	< 0.01	< 0.01	± 29.6	± 2.2	± 0.1
Outside		13.2	38.1	1166 *	10.3	0.95

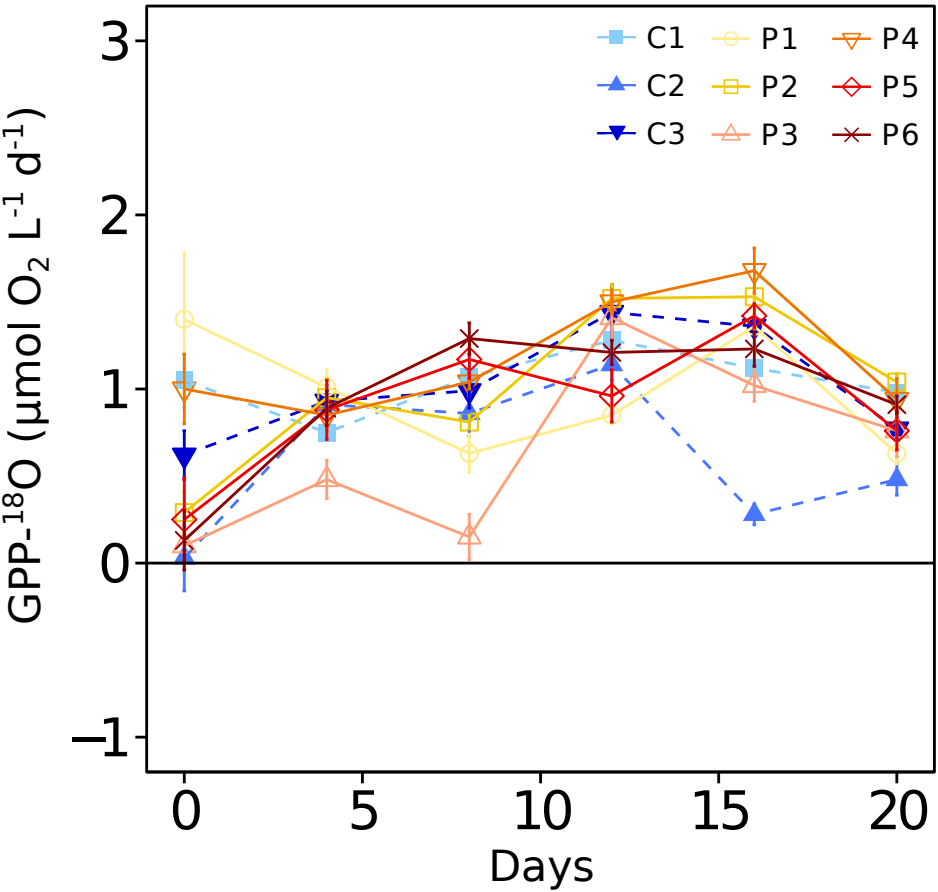
* measured on February 19th

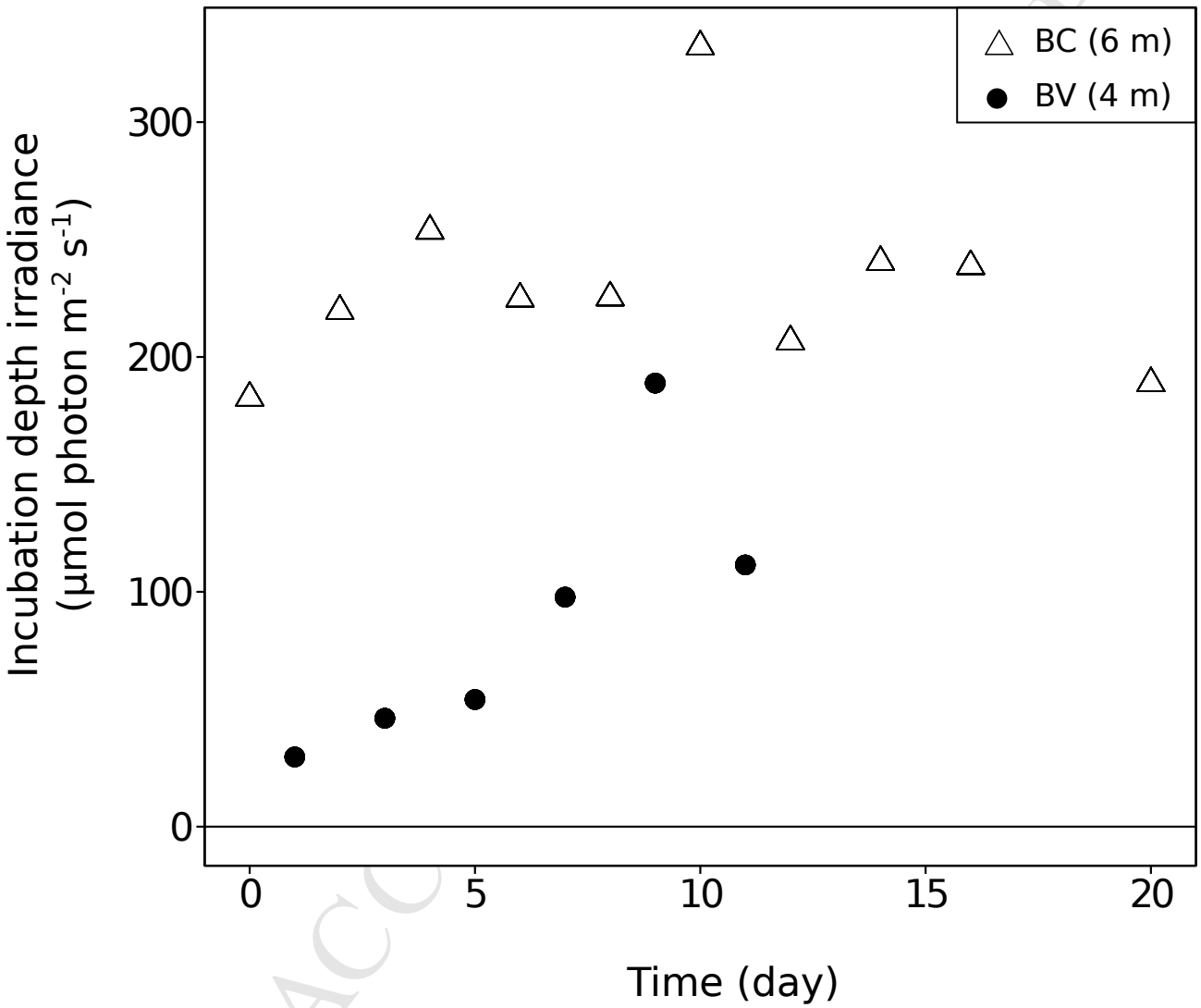
Table 3: Effects of ocean acidification as observed during previous mesocosm experiments under different environmental conditions. The range in nitrogen (NOx = nitrate + nitrite), phosphate (DIP) and chlorophyll *a* (chl *a*) concentrations as well as temperature (T) and the main phytoplankton groups are presented. LOD: below detection limit and ND: not determined.

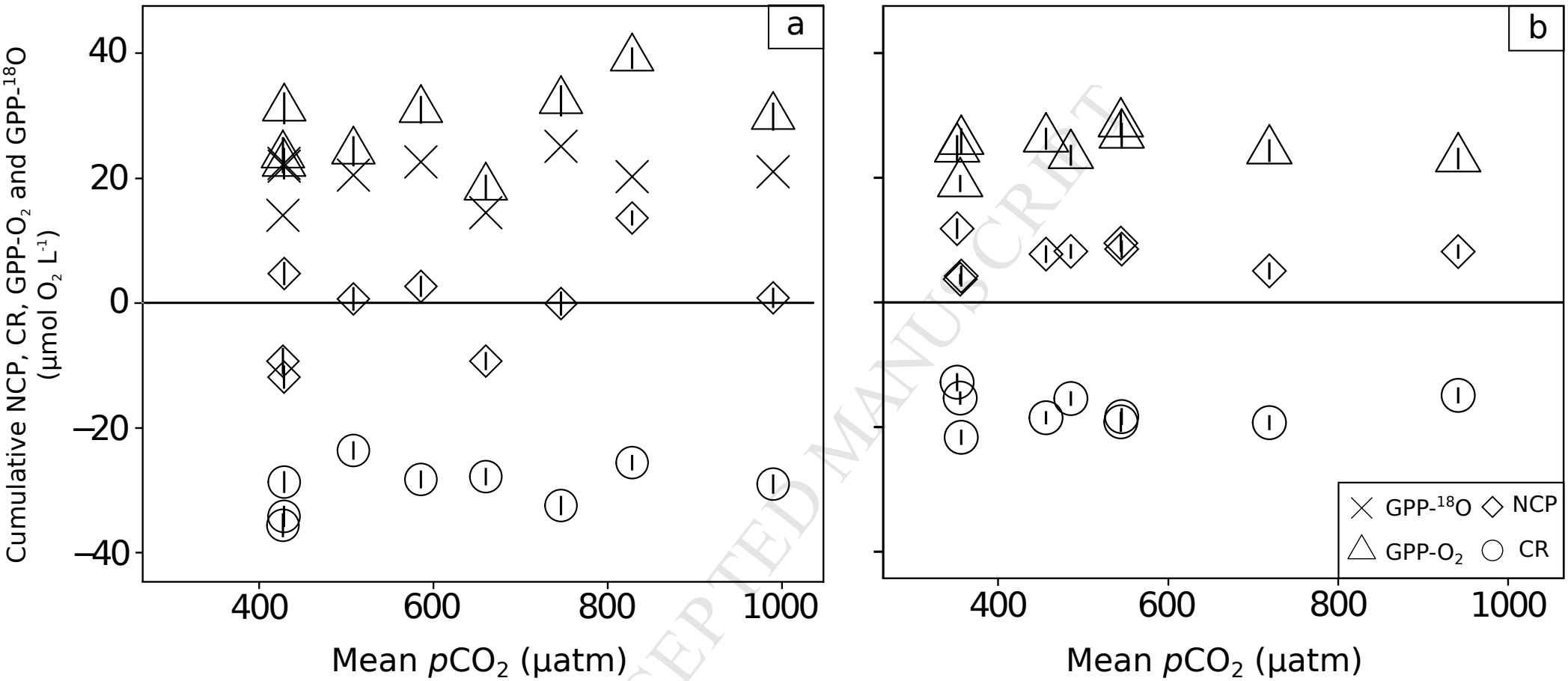
Experiment, location and year	T (°C)	NO_x (µmol L^{-1})	DIP (µmol L^{-1})	chl *a* (µg L^{-1})	Main phytoplankton group	Effect on metabolic rates or main result	Reference
Low nutrient concentrations							
Bay of Calvi, NW Mediterranean (2012)	21.5 to 24.5	< 0.04	< 0.01	0.04 to 0.19	Haptophytes and cyanobacteria	No effect on community metabolism	this study; Gazeau et al. (in prep, this issue)
Bay of Villefranche, NW Mediterranean (2013)	13 ± 0.5	< 1.2	< 0.01	0.36 to 1.27	Haptophytes and cryptophytes	No effect on community metabolism	this study; Gazeau et al. (in prep, this issue)
Nutrient addition							
PeECE I Bergen (2001)	10 to 13	LOD to 17	LOD to 0.5	1 to 12.5	Temporal shift from *Synechococcus* spp. to *E. huxleyi*	No effect on particulate organic matter production	Delille et al. (2005)
PeECE II Bergen (2003)	8 to 10	LOD to 9	LOD to 0.5	0.2 to 4.2	Temporal shift from *E. huxleyi* to diatoms	Small species more affected, no effects on metabolic rates	Engel et al. (2008); Egge (unpublished data); Engel (unpublished data)
PeECE III Bergen (2005)	9 to 11.5	LOD to 15	LOD to 0.6	1.5 to 13	Temporal shift from diatoms and *E. huxleyi* to flagellates	Increase in primary production (^{14}C 24 h incubation) but no	Egge et al. (2009)

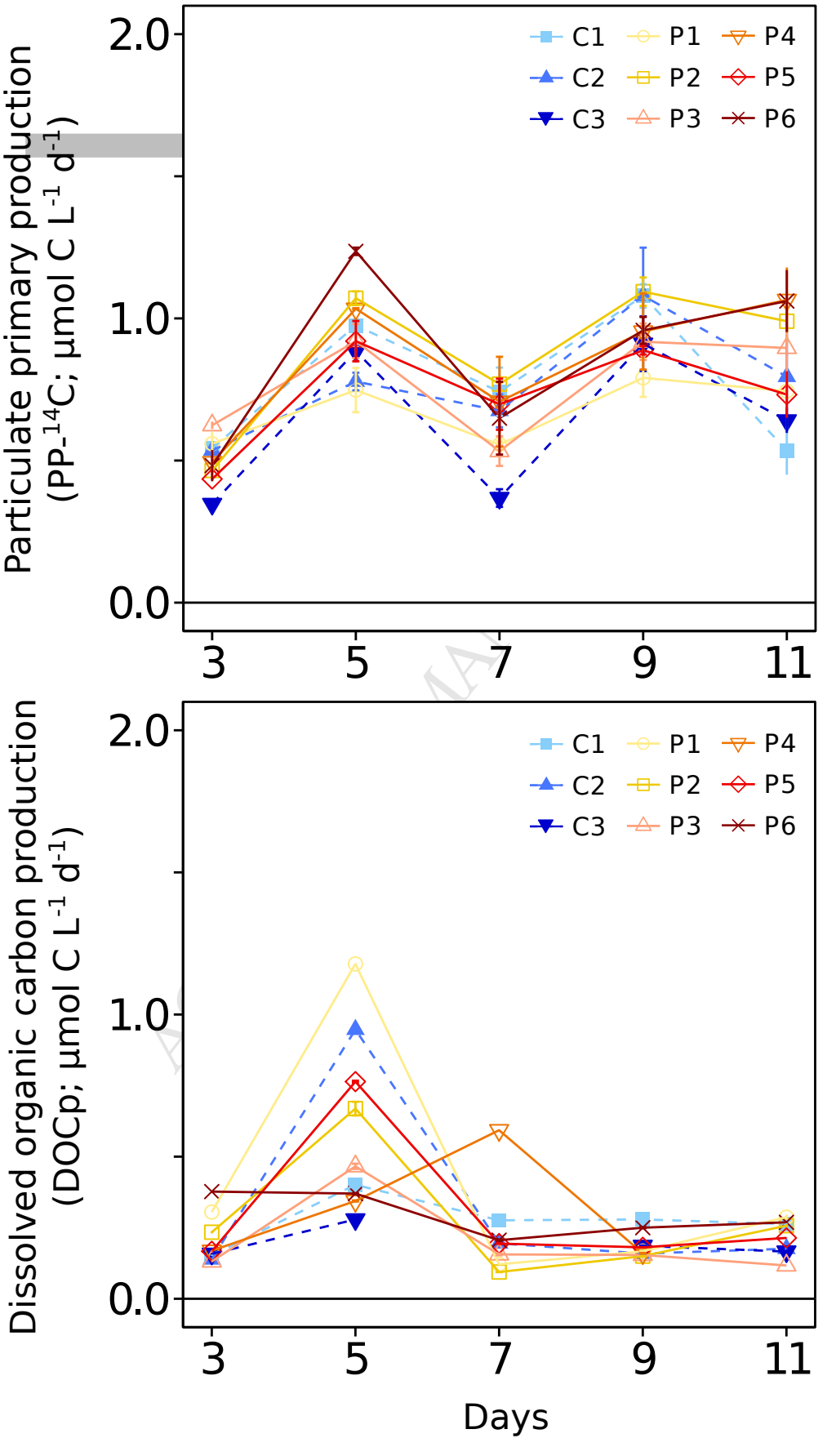
						effect on net community production (O_2)	
Jangmok Bay Korea (2008)	-	41 to LOD	2.5 to LOD	0.8 to 37	Diatoms dominated and some dinoflagellates in post-bloom	Decrease POC and increase DOC production. Increase light utilization but not reflected on primary production.	Kim et al. (2011); Kim et al. (2013)
Svalbard (2010)	2 to 5.5	0.1 to 5.5	0.09 to 0.4	0.22 to 2.7	Haptophytes and mixotrophes	Respectively no and negative effect on net community production O_2 and ^{13}C methods, for whole period. Positive effect on primary production based ^{14}C 24 h incubation	Tanaka et al. (2013); de Kluijver et al. (2013); Engel et al. (2013)


a
b
c
d
e
f
NCP (µmol L-1 d-1)
CR (µmol L-1 d-1)
GPP (µmol L-1 d-1)
Days
Days
C1
C2
C3
P1
P2
P3
P4
P5
P6




BC (6 m)
BV (4 m)
Incubation depth irradiance
(µmol photon m-2 s-1)
300
200
100
0
0
5
10
15
20
Time (day)




Particulate primary production
(PP-14C; µmol C L-1 d-1)
2.0
1.0
0.0
3
5
7
9
11
C1
C2
C3
P1
P2
P3
P4
P5
P6
Dissolved organic carbon production
(DOCp; µmol C L-1 d-1)
2.0
1.0
0.0
3
5
7
9
11
Days
C1
C2
C3
P1
P2
P3
P4
P5
P6