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First mesocosm experiments to study the impacts of ocean acidification on plankton communities in the NW Mediterranean Sea (MedSeA project)

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Abstract

There is a growing international interest in studying the effects of ocean acidification on plankton communities that play a major role in the global carbon cycle and in the consumption of atmospheric CO₂ via the so-called biological pump. Recently, several mesocosm experiments reported on the effect of ocean acidification on marine plankton communities, although the majority were performed in eutrophic conditions or following nutrient addition. The objective of the present study was to perform two mesocosm experiments in the oligo- to meso-trophic Northwestern Mediterranean Sea during two seasons with contrasting environmental conditions: in summer 2012 in the Bay of Calvi (Corsica, France) and in winter 2013 in the Bay of Villefranche (France). This paper describes the objectives of these experiments, the study sites, the experimental set-up and the environmental and experimental conditions during the two experiments. The 20-day experiment in the Bay of Calvi was undoubtedly representative of summer conditions in the Northwestern Mediterranean Sea with low nutrient and chlorophyll *a* concentrations, warm waters and high surface solar irradiance. In contrast, the winter experiment, which was reduced to 12 days because of bad weather conditions, failed to reproduce the mesotrophic conditions typical of the wintertime in this area. Indeed, a rapid increase in phytoplankton biomass during the acidification phase led to a strong decrease in nitrate concentrations and an unrealistic N and P co-limitation at this period of the year. An overview of the 11 papers related to this study and published in this special issue is provided.

1. Introduction

1.1. Context and objectives

During the last 150 years, human activities, through the combustion of fossil fuels (oil, gas and coal) and land use change, have led to an important release of carbon dioxide (CO₂) to the atmosphere. With an average annual uptake, during the period 2003 to 2012, of 2.5 ± 0.5 GtC (~26.3% of anthropogenic emissions; Le Quéré et al., 2014), the oceans substantially contribute towards slowing down the increase in atmospheric CO₂ concentrations, and therefore towards slowing global warming. However, this massive CO₂ input induces global changes in seawater chemistry referred to as "ocean acidification" because increased CO₂ lowers seawater pH (i.e., increases its acidity). On average, the pH in ocean surface waters has already decreased by 0.1 since the beginning of the industrial era, equivalent to an increased acidity of 26% (Ciais et al., 2013). According to recent projections, an additional decrease is expected by 2100, ranging from 0.06 to 0.32, equivalent to an increased acidity of 15 to 110%, depending on the considered CO₂ emission scenario (Ciais et al., 2013).

Over the past two decades, there has been a rapidly growing interest in the potential effects of ocean acidification and associated modifications of the carbonate chemistry on marine pelagic and benthic organisms (Kroeker et al., 2013). Early laboratory experiments mostly focused on the response of single species to ocean acidification (see Riebesell and Tortell, 2011 for a comprehensive review), therefore neglecting the influence of competitive and trophic interactions on single species responses. It has become more and more evident that experimentations at the community level are essential if we are to understand how ocean acidification will impact diversity and functioning of marine ecosystems. Natural high-CO₂ environments can provide crucial information on the effects on ocean acidification on trophic and competitive interactions. However, the number of such sites is limited and does not allow

testing for the effects of ocean acidification on a large variety of communities and ecosystems. Moreover, because of high spatial and temporal variability in pH in those peculiar environments, it is difficult to determine a reliable dose-response relationship, and eventually identify thresholds or tipping points. Finally, while these natural laboratories have provided very important insights on the sensitivity of benthic communities to ocean acidification (e.g., Hall-Spencer et al., 2008), lateral advection prevents using these sites to study plankton communities. During the last decade, some ocean acidification experiments have been performed at the community level by means of pelagic mesocosms. Mesocosms are defined as experimental enclosures from 1 thousand to several thousands of litres that allow the maintenance of natural communities under close-to-natural conditions (Riebesell et al., 2013a). They have been increasingly used in both aquatic and terrestrial ecology, especially in the framework of research on anthropogenic disturbances (Stewart et al., 2013).

Regarding ocean acidification research, the first mesocosm experiment was conducted in 2001 as part of the Pelagic Ecosystem CO₂ Enrichment (PeECE; Engel et al., 2005). During this experiment, three $p\text{CO}_2$ levels (glacial: 180, present: 370 and year-2100: 700 μatm) were tested in the coastal North Sea (Bergen, Norway), and a coccolithophore bloom was initiated by addition of nitrate and phosphate. Main outcomes from this study were (1) resilience of net community production and decrease of net community calcification and organic matter export with increasing $p\text{CO}_2$ (Delille et al., 2005) and (2) changes in the uptake stoichiometry and cell-specific growth rates (Engel et al., 2005). This experiment was repeated in 2003 (PeECE II; see Grossart et al., 2006) and in 2005 (PeECE III; see Riebesell et al., 2008), generally proving the suitability of these mesocosm techniques to study the effect of ocean acidification on plankton communities, but also highlighting some discrepancies between the responses observed during the three experiments (e.g., no effect on net community production during PeECE I: Delille et al., 2005; increase in carbon drawdown

during PeECE III: Riebesell et al., 2007). Other experiments using small mesocosms (3,000 L; Kim et al., 2008) were conducted in coastal waters of Korea, to test for the effect of ocean acidification whether in isolation (Kim et al., 2006) or in combination with ocean warming (Kim et al., 2013; Kim et al., 2011). Kim et al. (2006) showed, over a 14 days experiment with nutrient addition on day 8, no significant enhancement of organic carbon (dissolved and particulate) production between the CO₂ treatments although two diatom species increased in growth rate during the nutrient replete phase. In spring 2009, six large mesocosms were deployed in the coastal Western Baltic Sea and were enriched with nitrate and phosphate at the start of the experiment (Engel et al., 2014). In 2010, a similar experiment was conducted in the framework of the European project on Ocean Acidification (EPOCA; <http://www.epoca-project.eu>) when nine large free-floating mesocosms (ca. 50 m³; Riebesell et al., 2013a) were used to study the effects of ocean acidification, following a gradient approach, on plankton communities in coastal waters of the Arctic (Spitzbergen). Results showed significant sensitivity of this plankton community to ocean acidification with increased particulate and dissolved organic carbon production and autotrophic biomass, especially after nutrient addition, approximately half-way through during the experiment (Riebesell et al., 2013b).

All experiments presented above were performed in eutrophic conditions or with nutrient addition at the start or during the experiment, resulting in nitrate concentrations usually above 5 $\mu\text{mol L}^{-1}$ and phosphate concentrations around 0.5 $\mu\text{mol L}^{-1}$. However, there is an important diversity of oceanic provinces (Longhurst et al., 1995), from the less productive areas (ultra-oligotrophic) to very productive areas (eutrophic). About 50% of primary production on Earth takes place in the ocean although more than 60% of surface is associated with low productivity, termed oligotrophic areas. A decreased nutrient availability and expansion of low productivity regions are projected in a high CO₂ world, as enhanced

thermal stratification is expected to lead to surface layer nutrient depletion (Irwin and Oliver, 2009; Polovina et al., 2008). As nutrient availability is suspected to also have strong effects on the community response to ocean acidification (e.g., Hare et al., 2007), there is a need to evaluate the sensitivity of oligotrophic marine environments to this anthropogenic pressure. Recently, based on these considerations, a mesocosm experiment has been conducted in the summer Baltic Sea (Paul et al., 2015). This experiment showed significant positive effects of increased CO₂ concentrations on the biomass of small phytoplankton species without concomitant increased sedimentation rates but rather a shift of carbon partitioning towards more dissolved organic carbon.

The Mediterranean Sea is generally considered as an oligotrophic area but actually exhibits a gradient from mesotrophic-oligotrophic in the western basin to ultra-oligotrophic in the eastern basin (The Mermex group, 2011). It is considered a small-scale ocean with high environmental variability and steep physicochemical gradients within a restricted region (Bethoux et al., 1999). However, it presents higher salinity and total alkalinity levels than the open ocean, potentially allowing the absorption of more anthropogenic CO₂ than in this latter (CIESM, 2008). Based on satellite observations, it is estimated that the Mediterranean Sea, as a whole, acted as a small sink of CO₂ in the 2000s (9 Mt C yr⁻¹), with the western basin acting as a sink (11.5 Mt C yr⁻¹) and the eastern basin as a source (2.5 Mt C yr⁻¹; Taillandier et al., 2012).

The European project 'Mediterranean Sea Acidification under changing climate' (MedSeA; <http://medsea-project.eu>) was launched in 2011 with the objective to assess uncertainties, risks and thresholds related to Mediterranean acidification at organismal, ecosystem and economical scales. In the frame of this project, two large mesocosm experiments were performed in the Northwestern Mediterranean Sea during two seasons with contrasting environmental conditions. The experiments took place in June-July 2012 in the

Bay of Calvi (Corsica, France) and in February-March 2013 in the Bay of Villefranche (France). Both experiments gathered a multi-disciplinary team composed of 20-25 participants, originating from seven different countries (France, Spain, Greece, Italy, United Kingdom, Belgium and United States of America).

1.2. Study sites

1.2.1. Bay of Calvi

The Bay of Calvi (42°35' N, 8°45' E) is located on the northwest coast of Corsica (France) in the Mediterranean Sea (Fig. 1) and is bordered by the strong Western Corsican current. This oligotrophic area is classified as a “pristine site” where environmental disturbances caused by anthropogenic pressure are very low (Richir and Gobert, 2014). Most of the major Mediterranean coastal ecosystems are present in the area with healthy benthic (Gobert et al., 2014) and pelagic ecosystems associated with a high biodiversity.

In the Bay of Calvi, STARESO « STation de REcherches Sous marines et Océanographiques » is the marine research station established by the University of Liège (Belgium) in 1972 (<http://www.stareso.com>). It is currently managed by the French company STARESO SAS and also acts as a technical office toward communities and private customers in the field of marine environmental impact studies. The marine laboratory offers direct access to the sea, and facilitates investigations using divers, boats, and laboratories. Quality of the results due to this proximity is enhanced with continuous installation of different types of *in situ* probes directly connected to the laboratory (e.g., salinity, temperature, weather station). In parallel to these high frequency measurements, since 1970, time series of physical, chemical, and biological data (sampling at sea with automated systems and sensors deployed in the Bay, as well as *in situ* experiments) have been recorded.

The Bay of Calvi, a low-runoff system opened to the north, which has a narrow continental shelf with a steep canyon is influenced by oligotrophic waters of Atlantic origin and is under typical Mediterranean climate (no freezing temperatures in winter, dry and sunny summer with more than 350 sun hours in July, the most sunny month). Mean monthly precipitations range between 0 and 120 mm month⁻¹ in July and November respectively, with a yearly average of 50 mm yr⁻¹. During exceptional rainfalls (occurring mainly in spring and

autumn), freshwater inflows are significant, causing floods with a large quantity of suspended materials. The mean monthly air temperature ranges between 10 °C and 25 °C, in winter and summer respectively. Mean monthly surface water temperature ranges from a minimum of 12.4 ± 0.3 °C in February to a maximum of 26.6 ± 0.6 °C in August. Mean monthly PAR (photosynthetically active radiation) levels at 10 m below sea-surface present maximum values in July ($283 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and minimum values in March ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). External nutrient sources are limited and do not release the oligotrophy of surface waters during the winter-spring period. In spring, sea surface water warming results in a stratification that increase in the later summer months. The water column is stratified between May and October, and well mixed during the rest of the year. The mean depth of the thermocline is ca. 25-30 m. The height of the warm layer and the temperature gradient depend on meteorological events. Surface salinity tends to be about 37-38, only modified by extreme events (rainfalls, storms, exceptional upwelling from the open sea; e.g., Gobert, 2002).

A large collection of data focused on three primary producers: phytoplankton, macroalgae and seagrass (*Posidonia oceanica*) has been collected over the last 40 years. As a result, the seasonal and inter-annual dynamics of these major primary producers related to environmental parameters (temperature, wind, nutrient concentrations) are well known in the Bay (Bay, 1984; Champenois and Borges, 2012; Gobert et al., 2003; Lepoint et al., 2002). The Bay of Calvi is representative of an oligotrophic zone with low chlorophyll *a* concentration ($< 1 \mu\text{g Chl } a \text{ L}^{-1}$) except during the bloom period. Depending on the year, with large inter-annual variability, the principal bloom generally occurs in February, March or April. The transfer of nutrients from deeper layers, mainly controlled by hydrodynamic processes such as coastal upwellings and turbulent mixing generated by the wind, can be responsible for a plurimodal shape of plankton blooms (Goffart et al., 2002; Skliris et al.,

2001). Variations of surface temperature, salinity, nutrient and chlorophyll *a* concentrations in 2012 are shown in Fig. 1.

1.2.2. Bay of Villefranche

The Bay of Villefranche is located in the northern part of the Ligurian Sea (NW Mediterranean Sea; Fig. 1). Close enough to the coast and outside the Bay, a strong current moves along the French Riviera. This so-called northern current is cyclonic, running from east to west. Occasionally it enters the Bay, which results in continuous water renewal (Nival and Corre, 1976). To the north, the Bay is shallow (~13 m) and bathymetry then plunges rapidly to a depth of 80-100 m at the entrance making the transition to the open sea very sharp with no shelf. Thanks to a monitoring station at the entrance of the Bay ("Point B", 43°41.10' N, 7°18.94' E; 85 m water depth), all basic physical, hydrological, chemical and biological parameters are sampled weekly (<http://www.obs-vlfr.fr/Rade/>).

Several studies (e.g., Bonilla-Findji et al., 2010; Bustillos-Guzmán et al., 1995; Garcia-Comas et al., 2011; Vandromme et al., 2011) depicted hydrological seasons with coldest months in February and March (~13 °C over an homogenous water column) and warmest months in July, August and September (with stratified water and temperatures above 25 °C in the shallow surface mixed layer). According to Bustillos-Guzmán et al. (1995) and Bonilla-Findji et al. (2010), based on a stratification index, there are three types of periods differing with respect to water column stability (1) a summer stratified period, (2) mixed periods and (3) semi-mixed periods (for spring and fall-winter conditions). Transition regimes in between can occur over very short periods of time. Although there is a strong inter-annual variability, water stratification begins typically in March and is followed by a phytoplankton bloom. Nitrate maxima occur in winter, with values generally ranging from 0.5 to 2 $\mu\text{mol L}^{-1}$ (Vandromme et al., 2011). Chlorophyll *a* maxima occur in winter and spring, with values generally ranging from 0.35 to 0.60 $\mu\text{g L}^{-1}$. According to nitrate and chlorophyll *a* at Point B,

the entrance of the Bay of Villefranche is not as oligotrophic as the open ocean but still oligotrophic enough to represent well the open ocean (Sheldon et al., 1992). The site where the mesocosms were installed is characterized by an alternation of sandy spots and beds of the seagrass *Posidonia oceanica*, a very active species in the Bay. Variations of surface temperature, salinity, nutrient and chlorophyll *a* concentrations in 2013 are shown in Fig. 1.

2. Material and Methods

2.1. Large clean mesocosms

Nine mesocosms were deployed in the Bay of Calvi on June 18th 2012 (BC; 42°34'48" N, 8°43'33" E) and in the Bay of Villefranche on February 15th 2013 (BV; 43°41'49" N, 7°18'43" E).

The mesocosms were the same as the ones fully described in Guieu et al. (2014; Fig. S1). Mesocosms were made only with plastic materials. They consisted of large bags made of two 500 μm thick films of polyethylene mixed with vinyl acetate (EVA, 19%) with nylon meshing in between to allow maximum resistance and light penetration (produced by HAIKONENE KY, Finland). They were 2.3 m in diameter and 12.5 m in height for the cylindrical part, and 2.2 m for the conical part at the bottom for a theoretical final water volume when filled of $\sim 50 \text{ m}^3$ (Fig. S1). In order to preserve the structure of the surface waters and work in clean condition as much as possible while filling the bags, the bags were built in two parts: (1) a main cylinder (2.3 m diameter) ending with a diameter reduced to 1.5 m and (2) a bottom cone ending with a sediment trap (see description thereafter). In order to strengthen the main cylinder and to maintain the cylindrical shape for the duration of the experiment, the whole structure was rigidified thanks to five large rings made from polyethylene (PE) 40 mm diameter tubes. At the bottom of the main cylinder and at the top of

the cone, two polyvinyl chloride (PVC) circles (8 cm in width) were installed, thereby sandwiching the plastic bags.

Each bag was held thanks to a 'drum' structure, also made with PE tubes 40 mm in diameter. Each bag was hold at several points at the level of the upper ring and at the level of the ring just below the surface of the sea: this allowed us to avoid having tension applied directly to the bags. A group of three mesocosms (structure + bag) were assembled (named hereafter a cluster; Fig. 2) and towed by boat to the mooring site (25 m depth). Each cluster was moored using three anchor screws installed at 120° of each other and connected to sub-surface buoys, which were themselves linked to surface buoys (Fig. S2). Clusters were positioned at a minimum of 15 m from each other. The complete setup was a solid mooring capable of absorbing the sea swell while maintaining a supple and strong structure and ensuring that no tension was applied directly to the bags. Finally, in order to avoid atmospheric deposition, the top of the mesocosms were covered with ultra violet (UV)-transparent ethylene tetrafluoroethylene (ETFE) roofs (Fig. S1), except for periods of sampling during which the roofs were partially opened. These covers were elevated to ~10 cm above the top of the 'drum' holding the bags, allowing air to circulate in order to avoid a confinement effect in the trapped water.

The following days, the cylindrical bags were ballasted to fill them with ambient seawater. For that purpose, the opened bottom PVC plates of the bags were lowered carefully to 12 m depth. A 5 mm mesh-sized screen attached to the bottom plates excluded larger organisms. Four small ballasts were temporarily attached to the PVC bottom plate allowing the main cylinder to gently, but rapidly (\square 10 min), deploy vertically with the assistance of one diver. The deployment of all bags were performed on the same day within 8 h.

In order to minimize differences in starting conditions between enclosed water bodies in the nine mesocosm, they were left open at the bottom for 24 h allowing free exchange with

the surrounding waters and closed by divers as fast as possible (closing of all bags within 8 h). Closing involved screwing a conical bottom at the bottom after removing of the mesh. During the entire installation, the divers followed instructions to remain away from the inside areas of the bags in order to minimize disturbance of the captured waters, particularly from air bubbles.

2.2. Acidification and ^{13}C -addition

Among the nine deployed mesocosms, six were modified in terms of $p\text{CO}_2$ following a gradient slightly different in BC and BV (see Table 1), as a consequence of different ambient $p\text{CO}_2$ levels in the two sites at the two different periods (i.e. ~ 450 vs. $350 \mu\text{atm}$ in BC and BV, respectively). In BC, the six targeted elevated $p\text{CO}_2$ levels were P1: 550, P2: 650, P3: 750, P4: 850, P5: 1000 and P6: 1250 μatm . In BV, the levels were P1: 450, P2: 550, P3: 750, P4: 850, P5: 1000 and P6: 1250 μatm . As can be seen in Fig. 2, each cluster contained one control mesocosm and was accompanied by a medium and a high $p\text{CO}_2$ level (cluster 1: C1, P1, P4; cluster 2: C2, P2, P5 and cluster 3: C3, P3, P6). These elevated $p\text{CO}_2$ levels were reached by adding various volumes of CO_2 saturated seawater (Table 1). To do so, at both sites, seawater was pumped near the mesocosms and sieved onto a 5 mm mesh in order to remove large organisms. In a 600 L tank, pure CO_2 was actively bubbled for several minutes in order to achieve saturation (Fig. 3A) and water was transferred in 25 L plastic containers for addition to the mesocosms. From 75 to more than 300 L were added, depending on the targeted $p\text{CO}_2$ level (Table 1). A diffusing system (Fig. 3B, C) connected to 15 m tube (via a garden hose) and a membrane pump (Jabsco) was used to ensure a perfect mixing of this CO_2 -saturated seawater inside the mesocosms. The diffusing system consisted of an empty epoxy ball (15 cm diameter) in which many 1 mm holes have been drilled in order to disperse water as with a shower head. The diffusing system was slowly moved up and down during the injection over the entire depth of the mesocosms. In order to minimize the stress

induced by the addition of large quantities of acidic water, the acidification of the mesocosms was performed over four days.

During the last day of CO₂ saturated seawater addition, ¹³C sodium bicarbonate (NaH¹³CO₃; 99%) was added to each mesocosm to increase the δ¹³C signature of dissolved inorganic carbon pool (δ¹³C-DIC) to ca. 200‰ in BC and 100‰ in BV. In BC, on day 11, a second addition of NaH¹³CO₃ was performed to better constrain production rates and resulted in an enrichment of ca. 250‰. This was performed in order to follow the incorporation of carbon by the phytoplankton community and to trace its transfer to bacteria, zooplankton and sinking organic matter. Furthermore, the combination of ¹³C stable isotope labelling with biomarkers analyses was used to determine production rates at group-specific level (see Maugendre et al., in press, this issue-a, for more details). On June 24th 2012 (in BC) and February 21st 2013 (in BV), the targeted pCO₂ levels were reached and the experiment started (day 0).

2.3. Sampling timing and procedures

Sampling from the mesocosms and from the external environment (OUT) took place on a regular basis. Each cluster of three mesocosms was simultaneously sampled from a plastic platform by a team of two scientists (Fig. 3D). Overall, one sampling sequence duration was less than two hours. For most of the parameters and processes, depth-integrated (0-10 m) samplings were taken by using 3x integrating water samplers, IWS (HYDRO-BIOS©; Fig. 3E). The IWS units were hanged on a Kevlar cordage and downcasts were performed manually at a regular speed of 10 cm sec⁻¹ after rinsing it outside the mesocosms. The sampling schedule including the amount of water sampled every day is presented in Tables 2 and 3. The complete list of parameters and processes measured during the two experiments is presented in Table 4. All processes were measured based on samples taken before sunrise: 4:00 in BC and 5:00 in BV (local times). Processes influenced by light were

incubated *in situ* on an incubation line, moored near the mesocosms. 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). These incubations were performed for measuring net community production, gross primary production (^{18}O -technique; only in BC), carbon fixation (^{14}C ; Maugendre et al., in press, this issue-b), as well as nitrogen fixation (Rees et al., in press, this issue). Incubations for bacterial production (Celussi et al., in press, this issue) and community respiration (Maugendre et al., in press, this issue-b) 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, other samples were taken daily at 8:30 and every 2 days at 10:30 (see Tables 2 and 3). Among all parameters/processes considered during these two experiments, only water column DOC measurements were associated with very important contaminations, most likely due to the chosen sampling protocol by means of IWS.

Sampling for the microlayer was performed using a special tube of quartz (Ebling, unpublished) and was performed every 2 days on a single cluster (C3, P3, P6). The same cluster was also sampled by the mean of a Teflon pump (St-Gobain Performance Plastics) activated by the pressurized air from a diving tank, to collect surface (~15 cm) samples to characterize aerosols emissions (Schwier et al., 2015).

Weather permitting, conductivity-temperature-depth (CTD) casts were also performed on a daily basis in each mesocosm and in the external environment. A Sea-Bird Electronics (SBE) 19plusV2 system was operated with several probes placed in a laminar flow entrained by a pump – temperature and conductivity cells, dissolved oxygen sensor (SBE43) and fluorometer for chlorophyll *a* (WetLabs WetStar) – together with external sensors of strain gauge, upwelling irradiance (Biospherical QSP2300) and pH (SBE18). The system logged measurements on a regular data stream at 4Hz (4 scans per second). The CTD, set up without protection cage to avoid any steel contamination, was hanged on a Kevlar cordage and 12 m

depth downcasts were performed manually at a regular speed of 10 cm sec^{-1} after a soaking phase long enough to relax the thermal inertia of sensors. The data streams per mesocosm were processed by a unique methodology set up from Sea-Bird recommendations, and provided 0.25 m resolution continuous profiles for temperature, salinity, density, O_2 , chlorophyll *a* and pH. pH values expressed in the NBS scale were corrected and expressed on the total scale using values of dissolved inorganic carbon (C_T) and total alkalinity (A_T) based on integrated samples (see section 2.5.2).

Collection of sediment traps was performed by a diver (Fig. 3F, G) on a daily basis in BC (Table 2) and less regularly as a consequence of bad weather conditions in BV (Table 3). On each occasion and for each mesocosm, divers followed the same procedure: (1) hitting the cone of the mesocosms in case some sinking material was retained on the walls, (2) waiting for 15 minutes, (3) closing the collector (Fig. S3), (4) collecting the 250 mL flask screwed to the trap system, (5) immediately replacing the sampled flask by a new empty one and (6) opening the collector again. All mesocosms were sampled within 30 min. Back in the laboratory, samples were immediately preserved in a pH buffered formalin solution (5%).

In BC, a final zooplankton net haul (200 μm mesh size) was performed in each mesocosm at the end of the experiment. Unfortunately, in BV, on March 7th, a storm caused non-repairable damages to the bags and no zooplankton net haul could be performed. In BC, samples were immediately preserved in a pH buffered formalin solution (5%).

All data collected during the two experiments are freely available on Pangaea, Bay of Calvi: <http://doi.pangaea.de/10.1594/PANGAEA.810331> and Bay of Villefranche: <http://doi.pangaea.de/10.1594/PANGAEA.835117>.

2.4. Volume and air-sea fluxes measurements

The volume of water enclosed in each mesocosm was estimated following the procedure based on salt addition described by Czerny et al. (2013a). Since no nutrient

addition was performed during our experiment, in contrast, for instance, to the mesocosm experiment conducted in Spitzbergen (Riebesell et al., 2013b), there was no real need to estimate the volume of the bags at the start of the experiment. In order to avoid potential salt addition effects on the plankton communities trapped in the bags, it had been decided to estimate these volumes at the end of the experiments. This was done on July 15th and 16th in BC. Unfortunately, as a consequence of a storm that damaged the bags and forced stopping the experiment in BV on March 7th, volume measurements could not be performed. In BC, volume measurements were performed based on the addition of 25 L of a brine solution at a salinity of 79.77 to each mesocosm. The salinity of this solution was carefully quantified based on a calibration curve performed in the Laboratoire d'Océanographie de Villefranche in July 2012, following the procedure described by Czerny et al. (2013a). Briefly, a volume of 10 mL of this solution was added to 0.5, 1, 2, 10 and 20 L of seawater collected in BV and salinity was measured before and after addition of the brine on an autosalinometer (Guildline©). In BC, the brine solution was prepared in the same 600 L tank used for the preparation of CO₂-enriched seawater. The 25 L of brine were then added to the bags using the same diffusing system as for acidification. However, in contrast to CO₂-saturated water addition, the diffusing system was hauled only in the first 5 m of the bags in order to avoid the production of high-saline/high density water at the bottom of the bags. The volume of each bag was then calculated as a function of the increase in salinity, as measured by CTD profiles before and after brine addition. The volume of each bag was back calculated to the start of the experiment, assuming the mass of salt varied as a consequence of water removal (sampling) and water addition (acidification), following the equation:

$$V_{t-1} = \frac{V_t S_t + V_s S_t - V_a S_{OUT}}{S_{t-1}}$$

where V_{t-1} and V_t are the volumes at day t-1 and day t, respectively, S_t is the averaged salinity measured at day t, V_s is the volume of water sampled at day t, V_a is the amount of

water added and S_{OUT} is the salinity of the external environment, used for the preparation of the CO_2 -saturated water.

The volume of water lost via evaporation throughout the experiment was then calculated as:

$$E = V_{final} - V_{initial} + V_s - V_a$$

Air-sea gas exchanges were estimated in one bag (C1) during each experiment at day - 1 (last day of CO_2 -saturated water addition), following the procedure described in Czerny et al. (2013b). A volume of one litre of saturated N_2O solution was prepared by bubbling N_2O (Medical grade, Air Liquide, 98% pure) for two days in filtered seawater sampled at the vicinity of the mesocosms. This N_2O saturated solution was mixed with 25 L of seawater sampled from mesocosm C1 and added to the mesocosm using the diffusion system described earlier. Samples for nitrous N_2O concentrations were collected with the IWS every two days, distributed in 50 mL glass serum bottles, poisoned with 100 μ L of saturated $HgCl_2$ and sealed with butyl stoppers and aluminium caps.

The flux of N_2O (F_{N_2O}) was computed from the slope (α in $nmol L^{-1} d^{-1}$) of the linear regression between N_2O concentration ($nmol L^{-1}$) and time, based on three consecutive measurements, and the depth of mesocosm (d in m) according to:

$$F_{N_2O} = \alpha \times d$$

where F_{N_2O} is given in $\mu mol m^{-2} d^{-1}$.

The gas transfer velocity normalized to a Schmidt number of 600 (k_{600} in $cm h^{-1}$) was then computed according to:

$$k_{600} = F_{N_2O} / \Delta N_2O$$

where ΔN_2O is air-sea gradient of N_2O computed from the average dissolved N_2O from the three consecutive measurements, and N_2O at saturation based on N_2O atmospheric partial pressure and N_2O Henry's constant (Weiss and Price, 1980).

Each k_{600} value was related to the wind speed averaged for the period corresponding to the three consecutive measurements.

2.5. Analytical methods

2.5.1. Surface measurements

During the two experiments, surface photosynthetic active radiation (PAR) and atmospheric $p\text{CO}_2$ have been measured continuously, between June 26th and July 18th in BC and during the entire experiment in the BV. In BC, all the equipment was installed on the Revelatta lighthouse (42°34'59" N, 8°43'28" E) and on top of the Laboratoire d'Océanographie de Villefranche in BV (43°41'47" N, 7°18'26" E). Irradiance was measured by a LI-192SA quantum sensor (LI-COR©) connected to a LI-1400 (LI-COR©) data logger. Atmospheric $p\text{CO}_2$ was measured using a non-dispersive infrared gas analyser (LI-820, LI-COR©) connected to an air pump (LI-670 Flow Control Unit, LI-COR©) maintaining an air (dried using magnesium perchlorate) flow to the gas analyser of 1 L min⁻¹. The LI-820 CO₂ gas analyzer was calibrated every 4 days with a standard two-point calibration (0 and 375 ppm). The "375 ppm" point was obtained by flowing a (dried) gas mixture with a certified CO₂ molar fraction of $375.2 \pm 0.5\%$ (Deuste Steininger) and the "0" point by flowing the same gas mixture from which all the CO₂ was removed by means of soda lime. In BC, wind speed and direction were recorded with a THIES© anemometer deployed, by the University of Liège, on top of one of buildings of the Stareso station (at 11.8 m height) at a distance of about 400 m from the mesocosms. For the experiment in the BV, wind speed data (daily averages) were obtained from the Météo France station at the Nice-Côte d'Azur International Airport (43°39'55" N, 7°12'48" E).

2.5.2. Carbonate chemistry measurements

Seawater samples for total alkalinity (A_T ; 500 mL) measurements were collected every day in all mesocosms, filtered on GF/F membranes and analyzed within one day. A_T was

determined potentiometrically using a Metrohm® titrator (Titrando 888) and a glass electrode (Metrohm®, ecotrode plus) calibrated using first NBS buffers (pH 4.0 and pH 7.0, to check that the slope was Nernstian) and then using TRIS buffer solutions (salinity 35, provided by A. Dickson, Scripps university, USA). Triplicate titrations were performed on 50 mL sub-samples at 22 °C and A_T was calculated as described by Dickson et al. (2007). In BC, titrations of standard seawater provided by A. Dickson (batch 117) yielded A_T values within $1.6 \mu\text{mol kg}^{-1}$ of the nominal value (standard deviation = $1.2 \mu\text{mol kg}^{-1}$, $n = 41$). In BV, the same procedure with batch 122 yielded accuracies and precisions of 0.7 and $1.0 \mu\text{mol kg}^{-1}$ ($n = 28$). Seawater samples for total inorganic carbon (C_T ; 120 mL) measurements were collected every day in all mesocosms and directly poisoned with 20 μL of a saturated solution of mercury chloride (HgCl_2). C_T was determined on triplicate 1.2 mL subsamples using an inorganic carbon analyser (AIRICA, Marianda®, Kiel, Germany) coupled to an infrared gas analyser (LI-COR® 6262). This instrument was calibrated prior to sample analysis against a certified reference material provided by A. Dickson (batch 117 and 122 in BC and BV, respectively). In BC, the precision (SD) was $0.7 \mu\text{mol kg}^{-1}$ and the accuracy $0.5 \mu\text{mol kg}^{-1}$ ($n = 38$). In BV, precision and accuracy were respectively 0.8 and $0.6 \mu\text{mol kg}^{-1}$ ($n = 22$). All parameters of the carbonate chemistry were determined from C_T , A_T , temperature and salinity using the R package seacarb (Lavigne et al., 2014).

2.5.3. Nutrients and chlorophyll *a* measurements

In BC, samples for ammonium were immediately frozen and samples for silicate were stored at 4°C pending analysis. Analyses were performed on a Skalar autoanalyser of the University of Liège (Belgium) within one month after collection. In BV, samples for ammonium have been processed directly and measured by a colorimetric technique (Holmes et al., 1999) and a laboratory fluorometer (Trilogy). Samples for the determination of silicate

were stored at 4 °C and measured using an autoanalyser (AA3 HR, Seal Analytical) at the Laboratoire d'Océanographie de Villefranche. During both experiments, nitrate+nitrite and phosphate concentrations were determined using nanomolar techniques and a Liquid Waveguide Capillary Cell (LWCC; Louis et al., in press, this issue).

For pigment analysis (including chlorophyll *a*), two litres of sampled seawater were filtered onto GF/F. Filters were directly frozen in liquid nitrogen and stored at -80 °C pending analysis at the Laboratoire d'Océanographie de Villefranche (France). Filters were extracted at -20 °C in 3 mL methanol (100%), disrupted by sonication and clarified one hour later by vacuum filtration through GF/F filters. The extracts were rapidly analyzed (within 24 h) by high performance liquid chromatography (HPLC) with a complete Agilent Technologies system. The pigments were separated and quantified as described in Ras et al. (2008).

2.5.4. Nitrous oxide measurements

Concentrations of N₂O were determined at the University of Liège (Belgium) via the headspace equilibration technique (20 mL N₂ headspace in 50 mL serum bottles) and measured by gas chromatography (GC; Weiss, 1981) with electron capture detection with a SRI 8610C GC calibrated with CH₄:CO₂:N₂O:N₂ mixtures (Air Liquide Belgium©) of 1, 10 and 30 ppm CH₄ and of 0.2, 2.0 and 6.0 ppm N₂O, and using the solubility coefficients of Weiss and Price (1980), and using the equations according to Upstill-Goddard et al. (1996).

3. Results

3.1. Atmospheric conditions

Surface irradiance, wind speed and atmospheric *p*CO₂ levels during the two experiments are shown in Fig. 4. In BC, surface irradiance was rather constant during the entire experiment with minimal and maximal daily (sunrise to sunset) average values of 531 and 735 μmol photons m⁻² s⁻¹. Maximum irradiance levels (~1300-1400 μmol photons m⁻² s⁻¹

were reached at around 12:00 pm and the Light:Darkness (L:D) cycle was 16.5:7.5 and 16:8, respectively at the start and at the end of the experiment. In BV, minimal and maximal daily (sunrise to sunset) average values of 103 and 513 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were recorded with a L:D regime of 11.5:12.5, and maximal irradiance levels ($\sim 300\text{--}1100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) reached at 1:00 pm. Higher daily averaged wind speeds were recorded during the winter experiment in BV with very windy conditions experienced on day 8 that prevented sampling during that day, and even winder on day 12 and the following night (data not shown) that irreversibly damaged the bags. Background levels of $p\text{CO}_2$ were similar (~ 400 ppm) between the two sites with much larger variations due to local contaminations in the less remote Bay of Villefranche, close to the city of Nice on the French Riviera.

3.2. Volume and air-sea fluxes estimates

As can be seen in Table 5, the volume of the bags during the experiment in BC varied by less than 2% as a consequence of water addition and removal by sampling and evaporation. The estimated final volume of P6 differs importantly from the 8 other mesocosms. As all bags had the exact same physical dimensions and as P6 did not look more filled than the other mesocosms, a technical problem during salt addition appears as the most likely explanation for this difference. As such, we will assume, as a first approximation, a volume for this mesocosm as equal to the mean value of the other eight mesocosms.

During both experiments, the concentration of N_2O increased substantially after addition as compared to external conditions (39 vs. 8 nmol L^{-1} in BC and 220 vs. 9 nmol L^{-1} in BV; Fig. 5A, B). The k_{600} values ranged between 2.7 and 13.9 cm h^{-1} and were positively related to wind speeds, with higher values during the BV than the BC experiment (Fig. 5C). The k_{600} values were above those predicted from the Wanninkhof (1992) parameterization as a function of wind speed. This is probably related to artificially generated turbulence from

wave action on the enclosures of the mesocosms. We derived a parameterization for the mesocosms by assuming a constant offset from the Wanninkhof (1992) relationship:

$$k_{600} = 3.58 + 0.31 \times u^2$$

where k_{600} in cm h^{-1} and u is wind speed in m s^{-1} .

3.3. Initial conditions

Chemical conditions in the bags and in the external environment at the start of the two experiments (day 0, i.e. at the end of the acidification phase and 5 days after the closing of the bags) are shown in Table S1. In BC, all parameters except for carbonate chemistry were rather similar inside and outside the mesocosms with a salinity of ~ 38 and a temperature of $\sim 22^\circ\text{C}$. Nitrate (NO_3^-) concentrations were very low with an average value in the mesocosms of $49 \pm 15 \text{ nmol L}^{-1}$, very similar to the value measured outside (50 nmol L^{-1}). Phosphate (PO_4^{3-}) concentrations were also quite homogeneous between the different mesocosms averaging $23 \pm 4 \text{ nmol L}^{-1}$, a value slightly below the one measured outside (35 nmol L^{-1}). Ammonium (NH_4^+) concentrations were highly variable and ranged from $0.13 \text{ }\mu\text{mol L}^{-1}$ in P6 to $0.77 \text{ }\mu\text{mol L}^{-1}$ in P4. Although some technical issues prevented the measurements of silicate (Si) concentrations in several mesocosms, they appeared quite homogeneous in the four remaining mesocosms (average of $1.7 \pm 0.4 \text{ }\mu\text{mol L}^{-1}$) and slightly below outside levels ($1.9 \text{ }\mu\text{mol L}^{-1}$). As such strong variability was only visible for NH_4^+ , we believe these variations were due to small contaminations during sampling or storage. Dissolved oxygen (O_2) concentrations averaged $226 \pm 1 \text{ }\mu\text{mol L}^{-1}$ in the mesocosms, corresponding to a percentage of saturation of 103%. Levels of $p\text{CO}_2$ were similar in the three control mesocosms (C1, C2 and C3) and outside, above atmospheric equilibrium, at around $460 \text{ }\mu\text{atm}$. Levels in the six perturbed mesocosms (P1 to P6) were generally slightly above targeted values (see Table 1).

As in the experiment in BC, hydrological conditions in BV were similar inside and outside the mesocosms at levels of ~ 38.1 and 13.2 °C for salinity and temperature, respectively. However, in contrast to BC, nutrient concentrations, especially NO_3^- were clearly different at day 0 (i.e. five days after the closing of the bags) between inside and outside mesocosms. Indeed, while NO_3^- concentrations inside the bags averaged 128 ± 29 nmol L^{-1} , values outside were one order of magnitude higher (1.2 $\mu\text{mol L}^{-1}$). This rapid decrease in NO_3^- concentrations was most likely due to an uptake by phytoplankton during the acidification phase as it was concomitant to a significant increase in biomass in all mesocosms. Although outside concentrations also slightly declined (from 1.6 to 1.2 μM) during that period (Louis et al., in press, this issue), $[\text{NO}_3^-]$ were maintained at a higher level due to a winter-mixing situation and/or due to external input via atmospheric wet depositions (Migon, pers. comm.). PO_4^{3-} concentrations were similar inside and outside the mesocosms and below values measured in BC (average of 11 ± 2 nmol L^{-1}). Similarly, NH_4^+ and Si concentrations were rather homogeneous and lower than values measured in BC in summer 2012 (respectively 0.07 ± 0.01 and 1.2 ± 0.1 $\mu\text{mol L}^{-1}$). O_2 concentrations averaged 249 ± 1 $\mu\text{mol L}^{-1}$ in the mesocosms, corresponding to a percentage of saturation of 96%. $p\text{CO}_2$ conditions were similar in the three control mesocosms and outside, below atmospheric equilibrium, at around 350 μatm . Levels in the six perturbed mesocosms were close to targeted values (see Table 1).

Chlorophyll *a* concentrations were higher in BV than in BC (Table S2). In BC, chlorophyll *a* concentrations were lower in the mesocosms than outside (0.06 ± 0.007 vs. 0.12 $\mu\text{g L}^{-1}$). No samples were taken for HPLC analyses before day 0, but fluorometry data from CTD casts (data not shown) during the acidification period suggest a decrease of phytoplankton biomass in the mesocosms during the first few days after closure of the bags. Using modified CHEMTAX, concentrations of chlorophyll *a* equivalent of eight taxonomic

groups of phytoplankton were estimated (see Gazeau et al., *sbm*, this issue for more details). The phytoplankton community was dominated both outside and inside the bags by haptophyceae, representing $\sim 36 \pm 5\%$ of the chlorophyll *a* equivalent biomass. The second most important species were cyanobacteria that were more abundant outside than inside mesocosms. In BV, chlorophyll *a* concentrations were higher in the mesocosms than outside (1.1 ± 0.1 vs. $0.95 \mu\text{g L}^{-1}$). The composition of the autotrophic community was similar between inside and outside the bags and dominated by cryptophyceae ($26 \pm 1\%$ of chlorophyll *a* equivalent biomass), followed by haptophyceae ($21 \pm 1\%$) and pelagophyceae ($18 \pm 1\%$). In contrast to BC, cyanophyceae were not dominant in BV and represented only 3% of the chlorophyll *a* equivalent biomass.

3.4. Environmental and experimental conditions during the experiments

Temperature, salinity and O_2 profiles (in $\mu\text{mol L}^{-1}$ and in % of saturation) in the mesocosms and in the external environment (OUT) are shown in Fig. S4 to S7 and Fig. S8 to S11, respectively for BC and BV. In BC, temperature profiles were very similar between inside and outside the bags with a significant overall increase between the start and the end of the experiment. In the mesocosms and outside, averaged temperature levels increased on average from 22.1 ± 0.04 at day 0 to 24.2 ± 0.01 °C at day 20. At the start of the experiment, both inside and outside the bags, the water column was homogeneous and a stratification appeared at a depth of 2-3 m on day 5 and remained for few days. Stronger winds on day 8 broke this stratification and the water column remained homogeneous until the end of the experiment. In contrast to temperature, salinity did not evolve similarly during the experiment inside and outside the bags. While salinity variations in the Bay reflected circulation changes between the Bay and the open Mediterranean Sea, the increase of salinity observed inside the bags was a consequence of the isolation of the water mass and subsequent evaporation during the experiment. Note that salinity remained homogeneous in the water column, but dispersion

in between mesocosms significantly increased at the end of the experiment, with averaged salinity values ranging at day 20 from 38.11 in C3 to 38.22 in P2. This can be due to differences in evaporation rates as a consequence of different positions of mesocosms in each cluster toward wind bursts during the last week of the experiment. As for temperature, O₂ concentrations were very similar throughout the experiment, inside and outside mesocosms and, following the observed warming during the experiment, average values (mesocosms and OUT) decreased from 226 ± 1 at day 0 to 208 ± 1 $\mu\text{mol L}^{-1}$ at day 20, and remained above values at saturation.

In BV, temperature remained more or less stable and vertically homogeneous at ca. 13 °C during the experiment until the storm that damaged the bags. At that moment (day 13-14), the entrance of external water in the bags is clearly visible for all mesocosms, with significant decreases in both temperature and salinity in the first meters below surface. O₂ concentrations increased significantly during the first few days of the experiment (acidification phase) in relation to the increase in chlorophyll *a* and the decrease in NO₃⁻ concentrations. O₂ concentrations remained thereafter constant until the end of the experiment (day 12).

The addition of CO₂-saturated water to the perturbed mesocosms, performed over four days, led to a gradual increase in *p*CO₂ and decrease in pH_T during both experiments (Fig. 6). Levels of *p*CO₂ reached at the end of the acidification phase were slightly above targeted levels, but were clearly different between the six perturbed mesocosms. For both experiments, *p*CO₂ and pH_T levels in control mesocosms remained similar to levels measured outside the mesocosms throughout the experiments. In BC, *p*CO₂ levels in P1 and P2 remained rather constant and well separated from each other during the entire experiment. In contrast, due to CO₂ degassing at the air-sea interface, *p*CO₂ levels in P5 reached the levels of P4 after 14 days of experiment. Following stronger winds on day 19, levels in P6 finally reached P4 and P5 levels by day 20. Declines in *p*CO₂ were much faster during the experiment in BV as a

consequence of stronger winds and sea surface turbulence. After 4 days of experiment (day 4), $p\text{CO}_2$ levels in P1 and P2 were similar and at day 6, P1, P2, P3 and P4 were not distinguishable anymore in terms of $p\text{CO}_2$. P5 and P6 remained well separated until the end of the experiment, although $p\text{CO}_2$ levels declined also sharply. During both experiments, A_T levels increased with time as a consequence of evaporation. In BC, the addition of ^{13}C -bicarbonate was responsible for a significant increase in A_T on day 1. Less labelled bicarbonate was added both on day 11 in BC and on day -1 in BV, and these additions did not lead to significant increases in A_T .

pH profiles acquired using the CTD and transformed to the total scale using integrated samples of A_T and C_T are presented in Fig. 7 and 8. In both sites, pH levels were very homogeneous in the water column.

In BC, variations in NH_4^+ were quite important and suggest contamination issues during sampling and/or storage (Fig. 9, upper panel). Due to this strong variability, no clear temporal pattern could be detected. In contrast, in BV, much lower concentrations were measured that decreased during the experiment for all mesocosms. In BC, silicate concentrations were rather homogeneous, lower than concentrations measured outside mesocosms and continuously decreased during the experiment (Fig. 9, central panel). In BV, concentrations were constant during the experiment, at levels generally lower than outside concentrations.

Chlorophyll *a* concentrations remained at very low levels in all mesocosms during the experiment in BC, well below concentrations found in the Bay (OUT; Fig. 9 lower panels). Maximal values in BC were reached on day 14 and were, on average between the nine mesocosms, $0.09 \pm 0.001 \mu\text{g L}^{-1}$. In BV, concentrations were more than one order of magnitude higher (ca. $1 \mu\text{g L}^{-1}$) and increased significantly after closing the bags and during the acidification phase, in all mesocosms. Concentrations in the mesocosms were on average

higher than levels measured outside, except during the last day of the experiment (day 12). After the initial increase, concentrations remained stable for three days and then decreased almost linearly until the end of the experiment.

4. Discussion

4.1. Brief evaluation of the experimental setup

The large clean mesocosms used during these two experiments have been previously deployed on two occasions in a Bay in Corsica in 2008 and 2010 to test for the effects of atmospheric deposition events onto the surface of oligotrophic marine waters (Guieu et al., 2014). Similar mooring and filling procedures than used during these previous studies have been considered during our experiments. Conditions in the nine mesocosms during the two experiments were similar for most parameters at the start of the experiments (Table S1 and S2) suggesting that the same water mass has been isolated in all mesocosms. In BC, on day 0, chlorophyll *a* concentrations were almost half the concentrations measured in the Bay. Particulate organic carbon concentrations and fluorometric data acquired using daily CTD profiles (data not shown) show that phytoplankton biomass decreased during the acidification phase in all mesocosms with important organic matter export in the first few days after closing the bags (Gazeau et al., *sbm*, this issue). In contrast, in BV, data show that chlorophyll *a* concentrations increased during the acidification phase, consuming a large proportion of available nutrients, notably nitrate, before the start of the experimental phase and leading to an unrealistic N and P co-limitation at this period of the year (Louis et al., *in press*, this issue). These initial conditions in terms of nutrient availability are clearly away from ambient conditions and suggest that our experimental set-up (i.e. four days acidification phase, no nutrient addition) was not adapted to create conditions that are fully representative of the

winter season in this area. A deeper discussion on this issue and suggestions to avoid and/or solve these problems in future experiments are provided in Maugendre et al. (sbm, this issue).

These mesocosm experimental facilities were used for the first time to mimic future $p\text{CO}_2$ conditions as projected for the end of the century, following various CO_2 emission scenarios. The experimental protocol followed during our experiments is similar to the one used by the KOSMOS (Kiel Off-Shore Mesocosms for Future Ocean Simulations) team in their past ocean acidification experiments in Svalbard or in the Baltic (Engel et al., 2014; Paul et al., 2015; Riebesell et al., 2013a). This protocol and a custom made diffusing system allowed the establishment of vertically homogeneous pH/ $p\text{CO}_2$ profiles (Fig. 7 and 8) and to successfully reach targeted levels with a maximal relative pH deviation between actual and targeted levels of only 17% (in BC, mesocosm P5).

No CO_2 additions were performed during the experiments to maintain contrasted CO_2 levels. This procedure was legitimate in BC where $p\text{CO}_2$ levels declined continuously only for the highest $p\text{CO}_2$ levels (P5 and P6). In contrast, in BV, as $p\text{CO}_2$ levels sharply declined leading to some overlaps between mesocosms, a second CO_2 addition would have been necessary. Unfortunately, the storm that damaged almost all mesocosms prevented such further addition.

During both experiments, while temperature levels remained very similar between mesocosms and the external environment, salinity levels increased during both experiments, especially in BC in summer, as a consequence of evaporation. As can be seen in Fig. S4 and S8, salinity levels slightly diverged during the course of the experiments, due to differences in evaporation rates (Table 5 for BC only). The experimental set-up used in our study that consisted in grouping three mesocosms together undoubtedly led to such experimental artefacts, by artificially creating differences in wind and wave exposure. It is very likely that, due to these differences, gas transfer velocities differed between mesocosms. Unfortunately,

this cannot be estimated based on our air-sea exchange estimates since only one mesocosm was enriched with N_2O ; this will prevent estimating carbon budgets during our experiments. Besides differences in terms of wind and wave exposure, it cannot be excluded that all mesocosms did not receive the same light dose during the experiment, especially at low sun angles (morning and evening). Since irradiance profiles have been measured only around mid-day, this cannot be properly evaluated. Anyway, considering all these potential shortcomings, we recommend that clustering mesocosms, although it provides non negligible logistical advantages, should be avoided in future deployments in the framework of ocean acidification studies.

4.2. Representativeness of the experiments with respect to Mediterranean seasonal variability

The Mediterranean Sea is considered an oligotrophic ocean. At the scale of the whole basin, the spatial means of chlorophyll *a* concentrations show a marked seasonal cycle, with the highest values in winter, from December to March ($0.25\text{-}0.40\ \mu\text{g L}^{-1}$) and a minimum (ca. $0.07\ \mu\text{g L}^{-1}$) from June to September (Bosc et al., 2004). The experiments were conducted from either sides of the Ligurian Sea in the western basin, the only area in the Mediterranean Sea characterized by the presence of a large bloom with a peak in late winter-early spring, i.e., usually starting in February and ending in April (D'Ortenzio and Ribera d'Alcalà, 2009) but with important inter-annual variability both in terms of intensity and starting date (Bosc et al., 2004). According to the conditions encountered during BC and BV experiments, one can determine the representativeness of the two experiments with respect to seasons. The BC experiment was undoubtedly representative of summer conditions in the Ligurian Sea with low nutrient concentrations, low chlorophyll *a* concentrations, warm waters and high surface solar irradiance typical of those during summer in the Ligurian Sea (see Bosc et al., 2004, their Fig. 4, 5 and 7). For the BV experiment, the situation appears less clear as there was no

clear bloom that year as evidenced by the chlorophyll *a* data obtained weekly at Point B in 2013 (with maximal surface chlorophyll concentrations at 5 m in April reaching only $1 \mu\text{g L}^{-1}$; Fig. 1). According to those weekly data, the chlorophyll *a* concentrations at 5 m at the entrance of the Bay were between 0.5 and $0.8 \mu\text{g L}^{-1}$ during the BV experiment. It has to be noted that our data averaged over 10 m were slightly higher inside the Bay on day 0 (February 21st; $[\text{Chl}a]_{\text{OUT}} = 0.95 \mu\text{g L}^{-1}$; Table S2) but in the absence of measurement that day at Point B we cannot conclude on a spatial difference between the two sites or short-time changes over the whole area. Nevertheless, at the time of the experiment, the meteorological conditions were typical of winter with low light (daily maximal irradiance $< 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), cold temperature ($\sim 13^\circ\text{C}$) and no water stratification. Nitrate concentrations were high ($[\text{NO}_3^-] = 1.16 \mu\text{mol L}^{-1}$) typical of winter situation (Louis et al., in press, this issue). The conditions encountered for this second experiment can be qualified as pre-bloom conditions. However, closure of mesocosms can lead to an artificial (different from what happens outside the mesocosm) increase in biomass coinciding with nutrient consumption. Indeed, a rapid increase in biomass during the acidification phase (day -4 to -1) in the mesocosms during the BV experiment was observed with maximal values on day 0 ($[\text{Chl}a] = 1.1\text{-}1.2 \mu\text{g L}^{-1}$). A similar increase in biomass was observed outside, but NO_3^- concentrations were maintained thanks to external inputs. This is further discussed in Louis et al. (in press, this issue). At the start of BV experiment, nutrients were thus depleted inside the mesocosms, finally close to the nutrients concentrations encountered during the BC experiment. Our experiments are thus both typical of oligo- to mesotrophic systems: BC with summer oligotrophic environmental conditions and BV with winter mesotrophic environmental conditions although with low availability in both N and P.

4.3. Presentation of the special issue

In order to present data obtained during these two experiments on the effect of ocean acidification in a low nutrient low chlorophyll (LNLC) area, it has been chosen to group papers in a special issue. Besides the present paper that has the objective to describe in details the general set-up and the environmental and experimental conditions during the two experiments, the other 11 manuscripts cover various aspects of the chemistry, biology and their relationship to carbonate chemistry.

- Louis et al. (in press, this issue) present the evolution of nutrients determined with nanomolar techniques (nitrate and phosphate, along with their speciation between organic and inorganic forms, and dissolved iron) during both experiments.

- Gazeau et al. (sbm, this issue) report on the dynamics of particulate organic matter concentrations and export as well as on the dynamics of the autotrophic community based on high performance liquid chromatography (HPLC) measurements using modified CHEMTAX, and as measured by flow cytometry.

- Celussi et al. (in press, this issue) describe the abundance of planktonic prokaryotes, their activity both at the cell level and from a bulk point of view, and their potential to degrade several simple organic compounds (polysaccharides, polypeptides, lipids, chitin and phosphorilated molecules).

- Tsiola et al. (in press, this issue) focus on the importance of lysogeny as a key indicator of environmental status under the impact of ocean acidification. In BV, both lytically infected and lysogenic cells were determined in response to increased $p\text{CO}_2$ based on a viral reduction approach. □

- As planktonic calcifiers are potentially negatively affected by ocean acidification, sampling efforts have been dedicated to the study of the coccolithophores community composition with results presented in Oviedo et al. (in press, this issue).

- A focus on zooplankton has only been made during the experiment in BC (Zervoudaki et al., sbm, this issue) with the objective to determine the abundance and taxonomic composition of the mesozooplankton, to monitor eggs and nauplii stock and estimate the feeding rates of dominant copepod species at different $p\text{CO}_2$ levels.
- Bourdin et al. (in press, this issue) provide information on transparent exopolymeric particles (TEP) temporal variations in abundance, production and aggregation as well as a detailed description of the dynamics of their precursors in BV.
- Maugendre et al. (in press, this issue-b) study the effect of increasing $p\text{CO}_2$ levels on planktonic metabolism by means of incubations and several techniques (oxygen light-dark, ^{14}C labelling in BV and ^{18}O labelling in BC).
- In addition to measuring metabolic rates, at both sites, the flow of carbon within the plankton community is investigated using ^{13}C -labelling studies coupled with biomarkers analysis (Maugendre et al., in press, this issue-a).
- During the summer experiment in BC, Rees et al. (in press, this issue) investigate the relationship between ocean acidification, nitrogen fixation rate and the diazotrophic community composition.
- Finally, Maugendre et al. (sbm, this issue) review the current knowledge on the effects of ocean acidification on plankton communities, synthesise the new knowledge arising from the special issue and provide guidelines for future research.

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6. References

- Bay, D., 1984. A field-study of the growth dynamics and productivity of *Posidonia oceanica* (L) Delile in Calvi Bay, Corsica. *Aquatic Botany* 20, 43-64.
- Bethoux, J.P., Gentili, B., Morin, P., Nicolas, E., Pierre, C., Ruiz-Pino, D., 1999. The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. *Progress in Oceanography* 44, 131-146.
- Bonilla-Findji, O., Gattuso, J.P., Pizay, M.D., Weinbauer, M.G., 2010. Autotrophic and heterotrophic metabolism of microbial planktonic communities in an oligotrophic coastal marine ecosystem: seasonal dynamics and episodic events. *Biogeosciences* 7, 3491-3503.
- Bosc, E., Bricaud, A., Antoine, D., 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations. *Global Biogeochemical Cycles* 18.
- Bourdin, G., Gazeau, F., Kerros, M.E., Marro, S., Pedrotti, M.L., in press, this issue. Dynamics of transparent exopolymeric particles and their precursors during a mesocosm experiment: impact of ocean acidification. *Estuarine, Coastal and Shelf Science*.
- Bustillos-Guzmán, J., Claustre, H., Marty, J.C., 1995. Specific phytoplankton signatures and their relationship to hydrographic conditions in the coastal Northwestern Mediterranean Sea. *Marine Ecology Progress Series* 124, 247-258.

- Celussi, M., Malfatti, F., Franzo, A., Gazeau, F., Giannakourou, A., Pitta, P., Tsiola, A., Del Negro, P., in press, this issue. Ocean acidification effect on prokaryotic metabolism tested in two diverse trophic regimes in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science*.
- Champenois, W., Borges, A.V., 2012. Seasonal and interannual variations of community metabolism rates of a *Posidonia oceanica* seagrass meadow. *Limnology and Oceanography* 57, 347-361.
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- CIESM, 2008. Impacts of acidification on biological, chemical and physical systems in the Mediterranean and Black Seas, Menton (France).
- Czerny, J., Schulz, K.G., Krug, S.A., Ludwig, A., Riebesell, U., 2013a. Technical Note: the determination of enclosed water volume in large flexible-wall mesocosms "KOSMOS". *Biogeosciences* 10, 1937-1941.
- Czerny, J., Schulz, K.G., Ludwig, A., Riebesell, U., 2013b. Technical Note: a simple method for air-sea gas exchange measurements in mesocosms and its application in carbon budgeting. *Biogeosciences* 10, 1379-1390.

- D'Ortenzio, F., Ribera d'Alcalà, M., 2009. On the trophic regimes of the Mediterranean Sea: a satellite analysis. *Biogeosciences* 6, 139-148.
- Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R.G.J., Frankignoulle, M., Borges, A.V., Riebesell, U., Gattuso, J.P., 2005. Response of primary production and calcification to changes of $p\text{CO}_2$ during experimental blooms of the coccolithophorid *Emiliana huxleyi*. *Global Biogeochemical Cycles* 19.
- Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 191pp.
- Engel, A., Piontek, J., Grossart, H.P., Riebesell, U., Schulz, K.G., Sperling, M., 2014. Impact of CO_2 enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms. *Journal of Plankton Research* 36, 641-657.
- Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J.P., Harlay, J., Heemann, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M.D., Rochelle-Newall, E., Schneider, U., Terbrueggen, A., Riebesell, U., 2005. Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid *Emiliana huxleyi* in mesocosm experiments. *Limnology and Oceanography* 50, 493-507.
- Garcia-Comas, C., Stemann, L., Ibanez, F., Berline, L., Mazzocchi, M.G., Gasparini, S., Picheral, M., Gorsky, G., 2011. Zooplankton long-term changes in the NW Mediterranean Sea: Decadal periodicity forced by winter hydrographic conditions related to large-scale atmospheric changes? *Journal of Marine Systems* 87, 216-226.
- Gazeau, F., Sallon, A., Pitta, P., Pedrotti, M.-L., Marro, S., Guieu, C., sbm, this issue. Limited impact of ocean acidification on phytoplankton community structure in an oligotrophic

environment: results from two mesocosm studies in the Mediterranean Sea. Estuarine, Coastal and Shelf Science.

- Gobert, S., 2002. Variations spatiale et temporelle de l'herbier à *Posidonia oceanica* (L.) Delile (Baie de La Revellata-Calvi-Corse), 207 pp. Université de Liège, Liège, Belgique. <http://hdl.handle.net/2268/62892>.
- Gobert, S., Chéry, A., Volpon, A., Pelaprat, C., Lejeune, P., 2014. The seascape as an indicator of environmental interest and quality of the Mediterranean benthos: the *in situ* development of a description index: The LIMA, in: Musard, O. (Ed.), Underwater Seascape, Switzerland: Springer International <http://hdl.handle.net/2268/162104>, pp. 273-287.
- Gobert, S., Kyramarios, M., Lepoint, G., Pergent-Martini, C., Bouqueneau, J.M., 2003. Variations at different spatial scales of *Posidonia oceanica* (L.) Delile beds: effects on the physico-chemical parameters of the sediment. Oceanologica Acta 26, 199-207.
- Goffart, A., Hecq, J.H., Legendre, L., 2002. Changes in the development of the winter-spring phytoplankton bloom in the Bay of Calvi (NW Mediterranean) over the last two decades: a response to changing climate? Marine Ecology Progress Series 236, 45-60.
- Grossart, H.P., Allgaier, M., Passow, U., Riebesell, U., 2006. Testing the effect of CO₂ concentration on the dynamics of marine heterotrophic bacterioplankton. Limnology and Oceanography 51, 1-11.
- Guieu, C., Dulac, F., Ridame, C., Pondaven, P., 2014. Introduction to project DUNE, a DUst experiment in a low Nutrient, low chlorophyll Ecosystem. Biogeosciences 11, 425-442.

- Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454, 96-99.
- Hare, C.E., Leblanc, K., DiTullio, G.R., Kudela, R.M., Zhang, Y., Lee, P.A., Riseman, S., Hutchins, D.A., 2007. Consequences of increased temperature and CO₂ for phytoplankton community structure in the Bering Sea. *Marine Ecology Progress Series* 352, 9-16.
- Holmes, R.M., Aminot, A., K  rouel, R., Hooker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 1801–1808
- Irwin, A.J., Oliver, M.J., 2009. Are ocean deserts getting larger? *Geophysical Research Letters* 36.
- Kim, J.H., Kim, K.Y., Kang, E.J., Lee, K., Kim, J.M., Park, K.T., Shin, K., Hyun, B., Jeong, H.J., 2013. Enhancement of photosynthetic carbon assimilation efficiency by phytoplankton in the future coastal ocean. *Biogeosciences* 10, 7525-7535.
- Kim, J.M., Lee, K., Shin, K., Kang, J.H., Lee, H.W., Kim, M., Jang, P.G., Jang, M.C., 2006. The effect of seawater CO₂ concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. *Limnology and Oceanography* 51, 1629-1636.
- Kim, J.M., Lee, K., Shin, K., Yang, E.J., Engel, A., Karl, D.M., Kim, H.C., 2011. Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions. *Geophysical Research Letters* 38.

- Kim, J.M., Shin, K., Lee, K., Park, B.K., 2008. *In situ* ecosystem-based carbon dioxide perturbation experiments: Design and performance evaluation of a mesocosm facility. *Limnology and Oceanography-Methods* 6, 208-217.
- Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19, 1884-1896.
- Lavigne, H., Epitalon, J.M., Gattuso, J.-P., 2014. seacarb: seawater carbonate chemistry with R. ran.r-project.org/package=seacarb.
- Le Quéré, C., Peters, G.P., Andres, R.J., Andrew, R.M., Boden, T.A., Ciais, P., Friedlingstein, P., Houghton, R.A., Marland, G., Moriarty, R., Sitch, S., Tans, P., Arneeth, A., Arvanitis, A., Bakker, D.C.E., Bopp, L., Canadell, J.G., Chini, L.P., Doney, S.C., Harper, A., Harris, I., House, J.I., Jain, A.K., Jones, S.D., Kato, E., Keeling, R.F., Klein Goldewijk, K., Körtzinger, A., Koven, C., Lefèvre, N., Maignan, F., Omar, A., Ono, T., Park, G.H., Pfeil, B., Poulter, B., Raupach, M.R., Regnier, P., Rödenbeck, C., Saito, S., Schwinger, J., Segschneider, J., Stocker, B.D., Takahashi, T., Tilbrook, B., van Heuven, S., Viovy, N., Wanninkhof, R., Wiltshire, A., Zaehle, S., 2014. Global carbon budget 2013. *Earth Syst. Sci. Data* 6, 235-263.
- Lepoint, G., Millet, S., Dauby, P., Gobert, S., Bouqueneau, J.M., 2002. Annual nitrogen budget of the seagrass *Posidonia oceanica* as determined by in situ uptake experiments. *Marine Ecology Progress Series* 237, 87-96.
- Longhurst, A., Sathyendranath, S., Platt, T., Caverhill, C., 1995. An estimate of global primary production in the ocean from satellite radiometer data. *Journal of Plankton Research* 17, 1245-1271.

Louis, J., Guieu, C., Gazeau, F., in press, this issue. Nutrient dynamics under different ocean acidification scenarios in a low nutrient low chlorophyll system: the Northwestern Mediterranean Sea. *Estuarine, Coastal and Shelf Science*.

Maugendre, L., Gattuso, J.-P., de Kluijver, A., Soetaert, K., van Oevelen, D., Middelburg, J.J., Gazeau, F., in press, this issue-a. Carbon-13 labelling studies show no effect of ocean acidification on Mediterranean plankton communities. *Estuarine, Coastal and Shelf Science*.

Maugendre, L., Gattuso, J.-P., Guieu, C., Gazeau, F., sbm, this issue. Impacts of ocean acidification on plankton communities, a synthesis of MedSeA mesocosm experiments and a meta-analysis. *Estuarine, Coastal and Shelf Science*.

Maugendre, L., Gattuso, J.P., Poulton, A.J., Dellisanti, W., Gaubert, M., Guieu, C., Gazeau, F., in press, this issue-b. No detectable effect of ocean acidification on plankton metabolism in the NW oligotrophic Mediterranean Sea: results from two mesocosm studies. *Estuarine, Coastal and Shelf Science*.

Nival, P., Corre, M.C., 1976. Annual variation of surface hydrology in Bay of Villefranche-sur-mer. *Annales De L'Institut Océanographique* 52, 57-78.

Oviedo, A.M., Ziveri, P., Gazeau, F., in press, this issue. Coccolithophore dynamics in response to increasing $p\text{CO}_2$ in Mediterranean oligotrophic waters. *Estuarine, Coastal and Shelf Science*.

Paul, A.J., Bach, L.T., Schulz, K.G., Boxhammer, T., Czerny, J., Achterberg, E.P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., Riebesell, U., 2015. Effect of elevated CO_2 on organic matter pools and fluxes in a summer Baltic Sea plankton community. *Biogeosciences* 12, 6181-6203.

- Polovina, J.J., Howell, E.A., Abecassis, M., 2008. Ocean's least productive waters are expanding. *Geophysical Research Letters* 35.
- Ras, J., Claustre, H., Uitz, J., 2008. Spatial variability of phytoplankton pigment distributions in the Subtropical South Pacific Ocean: comparison between in situ and predicted data. *Biogeosciences* 5, 353-369.
- Rees, A.P., Turk-Kubo, K., Al-Moosawi, L., Alliouane, S., Gazeau, F., Hogan, M.E., Zehr, J.P., in press, this issue. Ocean acidification impacts on nitrogen fixation in the coastal western Mediterranean Sea. *Estuarine, Coastal and Shelf Science*.
- Richir, J., Gobert, S., 2014. A reassessment of the use of *Posidonia oceanica* and *Mytilus galloprovincialis* to biomonitor the coastal pollution of trace elements: New tools and tips. *Marine Pollution Bulletin* 89, 390-406.
- Riebesell, U., Bellerby, R.G.J., Grossart, H.P., Thingstad, F., 2008. Mesocosm CO₂ perturbation studies: from organism to community level. *Biogeosciences* 5, 1157-1164.
- Riebesell, U., Czerny, J., von Brockel, K., Boxhammer, T., Budenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S.A., Lentz, U., Ludwig, A., Mucche, R., Schulz, K.G., 2013a. Technical Note: A mobile sea-going mesocosm system - new opportunities for ocean change research. *Biogeosciences* 10, 1835-1847.
- Riebesell, U., Gattuso, J.P., Thingstad, T.F., Middelburg, J.J., 2013b. "Arctic ocean acidification: pelagic ecosystem and biogeochemical responses during a mesocosm study" Preface. *Biogeosciences* 10, 5619-5626.

- Riebesell, U., Schulz, K.G., Bellerby, R.G.J., Botros, M., Fritsche, P., Meyerhofer, M., Neill, C., Nondal, G., Oeschlies, A., Wohlers, J., Zollner, E., 2007. Enhanced biological carbon consumption in a high CO₂ ocean. *Nature* 450, 545-U510.
- Riebesell, U., Tortell, P.D., 2011. Effects of ocean acidification on pelagic organisms and ecosystems, in: Gattuso, J.-P., Hansson, L. (Eds.), *Ocean acidification*. Oxford University Press, Oxford, pp. 99-121.
- Schwier, A.N., Rose, C., Asmi, E., Ebling, A.M., Landing, W.M., Marro, S., Pedrotti, M.L., Sallon, A., Iuculano, F., Agusti, S., Tsiola, A., Pitta, P., Louis, J., Guieu, C., Gazeau, F., Sellegri, K., 2015. Primary marine aerosol emissions from the Mediterranean Sea during pre-bloom and oligotrophic conditions: correlations to seawater chlorophyll a from a mesocosm study. *Atmospheric Chemistry and Physics* 15, 7961-7976.
- Sheldon, R.W., Rassoulzadegan, F., Azam, F., Berman, T., Bezanson, D.S., Bianchi, M., Bonin, D., Hagstrom, A., Lavalpeute, M., Neveux, J., Raimbault, P., Rivier, A., Sherr, B., Sherr, E., Vanwambeke, F., Wikner, J., Wood, A.M., Yentsch, C.M., 1992. Nanoplankton and picoplankton growth and production in the bay of Villefranche-sur-mer (NW Mediterranean). *Hydrobiologia* 241, 91-106.
- Skirris, N., Elkalay, K., Goffart, A., Frangoulis, C., Hecq, J.H., 2001. One-dimensional modelling of the plankton ecosystem of the north-western Corsican coastal area in relation to meteorological constraints. *Journal of Marine Systems* 27, 337-362.
- Stewart, R.I.A., Dossena, M., Bohan, D.A., Jeppesen, E., Kordas, R.L., Ledger, M.E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J.B., Suttle, B., Thompson, R., Trimmer, M., Woodward, G., 2013. Mesocosm Experiments as a Tool for Ecological Climate-

- Change Research, in: Woodward, G., Ogorman, E.J. (Eds.), *Advances in Ecological Research: Global Change in Multispecies Systems*, Pt 3, pp. 71-181.
- Taillandier, V., D'Ortenzio, F., Antoine, D., 2012. Carbon fluxes in the mixed layer of the Mediterranean Sea in the 1980s and the 2000s. *Deep-Sea Research Part I-Oceanographic Research Papers* 65, 73-84.
- The Mermex group, 2011. Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. *Progress in Oceanography* 91, 97-166.
- Tsiola, A., Pitta, P., Giannakourou, A., Bourdin, G., Maugendre, L., Pedrotti, M.L., Gazeau, F., in press, this issue. Ocean acidification and viral replication cycles studied during a mesocosm experiment in the NW Mediterranean Sea. *Estuarine, Coastal and Shelf Science*.
- Upstill-Goddard, R.C., Rees, A.P., Owens, N.J.P., 1996. Simultaneous high-precision measurements of methane and nitrous oxide in water and seawater by single phase equilibration gas chromatography. *Deep-Sea Research Part I-Oceanographic Research Papers* 43, 1669-1682.
- Vandromme, P., Stemmann, L., Berline, L., Gasparini, S., Mousseau, L., Prejger, F., Passafiume, O., Guarini, J.M., Gorsky, G., 2011. Inter-annual fluctuations of zooplankton communities in the Bay of Villefranche-sur-mer from 1995 to 2005 (Northern Ligurian Sea, France). *Biogeosciences* 8, 3143-3158.
- Wanninkhof, R., 1992. Relationship between wind speed and gas exchange over the ocean. *Journal of Geophysical Research* 97, 7373-7382.

Weiss, R.F., 1981. Determinations of carbon-dioxide and methane by dual catalyst flame ionization chromatography and nitrous-oxide by electron-capture chromatography. *Journal of Chromatographic Science* 19, 611-616.

Weiss, R.F., Price, B.A., 1980. Nitrous oxide solubility in water and seawater. *Marine Chemistry* 8, 347-359.

Zervoudaki, S., Krasakopoulou, E., Moutsopoulos, T., Protopapa, M., Marro, S., Gazeau, F., sbm, this issue. Copepod response to ocean acidification in a low nutrient-low chlorophyll environment in the NW Mediterranean Sea. *Estuarine, Coastal and Shelf Science*.

Figure legends

Figure 1. Maps of the two study sites in France, the Bay of Calvi in Corsica and the Bay of Villefranche on the French Riviera. Hydrological (temperature and salinity) and biogeochemical (nutrients: nitrate + nitrite, NO_x ; silicate, Si; and phosphate, PO_4^{3-} and chlorophyll *a* concentrations) conditions in 2012 in surface waters of the Bay of Calvi (left panel) and in 2013 in surface waters of the Bay of Villefranche (right panel). For all plots, the corresponding experimental periods are identified by vertical dotted lines.

Figure 2. Schematic drawings of the mesocosm deployment (here in the Bay of Villefranche), showing the nine mesocosms grouped in clusters of three and showing the positioning of anchors and buoys.

Figure 3. Pictures and schemes showing the procedure used for acidifying and sampling the mesocosms as well as for collecting the sediment traps. A: schematic drawing of the tank used for the preparation of CO_2 -saturated seawater; B: picture of the diffusing system used to add CO_2 -saturated in the perturbed mesocosms; C: picture showing this diffusing system in a bag during addition of CO_2 -saturated water; D: picture of groups of two persons sampling the mesocosms from surface platforms; E: picture of the integrated water sampler (IWS); F and G: pictures showing the collection of sediment traps by a diver.

Figure 4. Surface atmospheric conditions (surface irradiance: upper panels, daily averaged wind speed: central panels and partial pressure of CO_2 , $p\text{CO}_2$: lower panels) during the experiments in the Bay of Calvi in summer 2012 (left panels) and in the Bay of Villefranche in winter 2013 (right panels).

Figure 5. Nitrous oxide (N_2O) concentrations in the N_2O enriched mesocosm (C1) and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012 (A) and in the Bay of Villefranche in winter 2013 (B). Gas transfer velocities (k_{600}) are

plotted against wind speed during each experiment. The derived parameterization is compared with the Wanninkhof (1992) open-ocean relationship.

Figure 6. Carbonate chemistry conditions in the nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012 (left panels) and in the Bay of Villefranche in winter 2013 (right panels). Partial pressure of CO_2 ($p\text{CO}_2$, upper panels) and pH on the total scale (pH_T , central panels) were calculated using seacarb, based on dissolved inorganic carbon concentrations (C_T , not shown) and total alkalinity (A_T , lower panels), measured daily from depth-integrated (0-10 m) samples. Vertical dotted lines show the start of the respective experiments (day 0 = d0). Targeted $p\text{CO}_2$ levels for perturbed mesocosms are indicated.

Figure 7. Profiles of pH on the total scale in the nine mesocosms (C1, C2, C3: control mesocosms and P1 to P6: perturbed mesocosms) during the experiment in the Bay of Calvi in summer 2012. Vertical profiles were taken daily by means of a hand-operated CTD and were corrected based on calculated pH (on the total scale) values based on daily depth-integrated (0-10 m) samples for dissolved inorganic carbon (C_T) and total alkalinity (A_T) measurements. Linear gridding has been applied to smooth the observed daily variations (3-day moving average). Targeted $p\text{CO}_2$ levels for perturbed mesocosms are indicated.

Figure 8. Profiles of pH on the total scale in the nine mesocosms (C1, C2, C3: control mesocosms and P1 to P6: perturbed mesocosms) during the experiment in the Bay of Villefranche in winter 2013. Vertical profiles were taken daily by means of a hand-operated CTD and were corrected based on calculated pH (on the total scale) values based on daily depth-integrated (0-10 m) samples for dissolved inorganic carbon (C_T) and total alkalinity (A_T) measurements. Vertical dotted black lines indicate the end of the experiment. Linear gridding has been applied to smooth the observed daily

variations (3-day moving average). Targeted $p\text{CO}_2$ levels for perturbed mesocosms are indicated.

Figure 9. Depth-integrated (0-10 m) ammonium concentrations (NH_4^+ ; upper panels), silicate concentrations (Si; central panels) and chlorophyll *a* concentrations (lower panels) in the nine mesocosms and in the external environments during the experiment in the Bay of Calvi in summer 2012 (left panels) and in the Bay of Villefranche in winter 2013 (right panels). The vertical dotted line on the right panels shows the start of the experiment (day 0). Targeted $p\text{CO}_2$ levels for perturbed mesocosms are indicated.

Supplementary material for:

First mesocosm experiments to study the impacts of ocean acidification on plankton communities in the NW Mediterranean Sea (MedSeA project)

Gazeau, F., Sallon, A., Maugendre, L., Louis, J., Dellisanti, W., Gaubert, M., Lejeune, P., Gobert, S., Borges, A.V., Harlay, J., Champenois, W., Alliouane, S., Taillandier, V., Louis, F., Obolensky, G., Grisoni, J.-M., and Guieu, C.

Figure S1: Scheme showing the different parts of a mesocosm. See section 2.1 in the manuscript for more details.

Figure S2: Scheme of the mooring procedure.

Figure S3: Picture and schemes showing the conical sediment trap.

Figure S4: Temporal dynamics of salinity in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S5: Temporal dynamics of temperature (°C) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S6: Temporal dynamics of dissolved oxygen concentrations ($\mu\text{mol L}^{-1}$) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S7: Temporal dynamics of dissolved oxygen concentrations (as a percentage of saturation) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Calvi in summer 2012. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S8: Temporal dynamics of salinity in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Villefranche in winter 2013. For mesocosms, the vertical dotted black line indicates the end of the experiment. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S9: Temporal dynamics of temperature (°C) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Villefranche in winter 2013. For mesocosms, the vertical dotted black line indicates the end of the experiment. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S10: Temporal dynamics of dissolved oxygen concentrations ($\mu\text{mol L}^{-1}$) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Villefranche in winter 2013. For mesocosms, the vertical dotted black line indicates the end of the experiment. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Figure S11: Temporal dynamics of dissolved oxygen concentrations (as a percentage of saturation) in all nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Villefranche in winter 2013. For mesocosms, the vertical dotted black line

indicates the end of the experiment. Vertical profiles were acquired daily by hand-operated CTD. Linear gridding has been applied to smooth the observed daily variations (3-day moving average).

Table 1. Ambient partial pressure of carbon dioxide ($p\text{CO}_2$ in μatm) conditions in the three control mesocosms (C1 to C3) after the closing of the bags (day -4) and targeted $p\text{CO}_2$ in the six perturbed mesocosms (P1 to P6, in bold) during both experiments in the Bay of Calvi in summer 2012 and in the Bay of Villefranche in winter 2013. Volumes (in L) of CO_2 -saturated seawater added to each perturbed mesocosm are reported.

	C1	C2	C3	P1	P2	P3	P4	P5	P6
Bay of Calvi									
Ambient or targeted $p\text{CO}_2$	435	439	436	550	650	750	850	1000	1250
Added volume	-	-	-	75	150	200	225	270	330
Bay of Villefranche									
Ambient or targeted $p\text{CO}_2$	346	352	347	450	550	650	750	1000	1250
Added volume	-	-	-	85	130	170	200	260	300

Table 2. Sampling schedule and sampled volumes during the experiment in the Bay of Calvi in summer 2012. IWS: Integrated water sampling, CTD: Conductivity-Temperature-Depth profiles, S: Surface sampling and D: Diving for sediment trap collections (see text for details). Surface sampling was performed only in the cluster with mesocosms C3, P3 and P6. A net haul was performed on July 15th to sample zooplankton organisms (Zervoudaki et al., *sbm*, this issue).

Date	Phase	Day #	Local time	Sampled volume (L)
17/06	Mesocosms deployment	-7		
18/06	-	-6		
19/06	Bag closing	-5		
20/06		-4	8:00	IWS/CTD 5
21/06		-3	8:00	IWS/CTD 5
22/06	Acidification	-2	8:00	IWS/CTD 5
23/06		-1	8:00	IWS/CTD/D 5
24/06		0	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
25/06		1	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/25
26/06		2	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
27/06		3	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
28/06		4	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
29/06		5	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/25
30/06		6	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
01/07		7	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
02/07		8	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
03/07		9	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/25
04/07	Experiment	10	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
05/07		11	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
06/07		12	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
07/07		13	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/25
08/07		14	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
09/07		15	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
10/07		16	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
11/07		17	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/25
12/07		18	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
13/07		19	Bad weather – no sampling	
14/07		20	4:00/8:30/12:00	IWS/IWS/CTD/D 15/15
15/07		21	8:00	WP2 net haul/CTD
16/07	Volume measurements	22	8:00	CTD

Table 3. Sampling schedule and sampled volumes during the experiment in the Bay of Villefranche in winter 2013. IWS: Integrated water sampling, CTD: Conductivity-Temperature-Depth profiles, S: Surface sampling and D: Diving for sediment trap collections (see text for details). Surface sampling was performed only in the cluster with mesocosms C3, P3 and P6. (No net haul could be performed to sample zooplankton organisms at the end of the experiment).

Date	Phase	Day #	Local time	Sampled volume (L)
14/02	Mesocosms deployment	-7		
15/02	-	-6		
16/02	Bag closing	-5		
17/02		-4	8:00	IWS/CTD/D 5
18/02		-3	8:00	IWS/CTD/D 5
19/02	Acidification	-2	8:00	IWS/CTD/D 5
20/02		-1	8:00	IWS/CTD 5
21/02		0	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
22/02		1	5:00/8:30/12:00	IWS/IWS/CTD/D 15/15
23/02		2	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
24/02		3	5:00/8:30/12:00	IWS/IWS/CTD 15/15
25/02		4	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD 2/15/15
26/02		5	5:00/8:30/12:00	IWS/IWS/CTD 15/15
27/02		6	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD/D 2/15/15
28/02	Experiment	7	5:00/8:30/12:00	IWS/IWS/CTD 15/15
01/03	Bad weather – no sampling			
02/03		9	5:00/8:30/12:00	IWS/IWS/CTD/D 15/15
03/03		10	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD 2/15/15
04/03		11	5:00/8:30/12:00	IWS/IWS/CTD/D 15/15
05/03		12	8:00/8:30/10:30/12:00	S/IWS/IWS/CTD 2/15/15
06/03	Storm – End of the experiment			

Table 4. List of parameters and processes measured during the mesocosms experiments in the Bay of Calvi (BC) in summer 2012 and in the Bay of Villefranche (BV) in winter 2013. References providing details and results of these measurements are also reported. IWS: Integrated water sampling, CTD: Conductivity-Temperature-Depth profiles, S: Surface sampling (see text for details).

Parameters	Frequency	Sampling method	Time (local)	Volume (L)	Reference
Hydrography					
Salinity					
Temperature					
Photosynthetically active radiation (PAR)	Daily	CTD	14:00	-	This paper
Oxygen (O ₂)					
pH _T					
Carbonate chemistry					
Total alkalinity (A _T)				0.5	
Total dissolved carbon (C _T)	Daily	IWS	8:30	0.12	This paper
Nutrients					
Ammonium (NH ₄ ⁺)				0.02	
Silicate (Si)	Daily	IWS	8:30	0.06	This paper
Nitrate (NO ₃ ⁻)				0.06	
Phosphate (PO ₄ ³⁻)	Daily	IWS	8:30	0.06	
Dissolved iron (DFe)				0.06	Louis et al. (in press, this issue)
Dissolved organic nitrogen/phosphorus (DON/DOP)	Every 2-4 days			0.12	
Organic and inorganic matter					
Particulate organic carbon (POC)	Daily	IWS	8:30	1.5	Gazeau et al. (sbm, this issue)

Particulate organic nitrogen (PON)				1.5	
Particulate phosphorus				1.5	
Transparent exopolymeric particles (TEP)	Every 2 days	IWS	10:30	2.0	BC: Iuculano (unpublished) BV: Bourdin et al. (in press, this issue)
cDOM			10:30		BC: Iuculano (unpublished)
Dissolved organic carbon (DOC)	Daily		8:30	0.06	Gazeau (unpublished)
Calcite in the fraction < 40 µm	Every 2 days	IWS	10:30 (BC) 8:30 (BV)	4-5 (BC) 2 (BV)	Oviedo (unpublished)
Diversity					
Pigments	Daily		8:30	2.0	
Direct microscope counting		IWS		0.5	Gazeau et al. (sbm, this issue)
Autotroph diversity	Every 2 days		4:00 (BC) 5:00 (BV)	0.01	
Heterotroph diversity	Every 2 days	IWS	4:00 (BC) 5:00 (BV)	0.01	Celussi et al. (in press, this issue)
Calcifying phytoplankton	Every 2 days	IWS	10:30 (BC) 8:30 (BV)	4-5 (BC) 2 (BV)	Oviedo et al. (in press, this issue)
Zooplankton diversity (only BC)	End of experiment	WP2 Net	14:00 (BC)	-	Zervoudaki et al. (sbm, this issue)
Microlayer					
Trace metals					
Dissolved organic carbon	Daily	Quartz cylinder	8:00	2	Ebling (unpublished)
Diversity (Flow cytometry)					

Primary Aerosols

Size distribution, hygroscopicity/CCN, organic content	Daily	S	8:30	3.0	Schwier et al. (2014)
Processes					
Nitrification	Every 2 days	IWS	4:00 (BC)	2.0	Al-Moosawi (unpublished)
Nitrate Uptake			5:00 (BV)	1.0	
Nitrogen fixation	Every 2 days	IWS	4:00 (BC)	2.0	BC: Rees et al. (in press, this issue) BV: Rees (unpublished)
Genes encoding enzymes involved in the fixation of atmospheric nitrogen (nif-H)	Every 4 days		5:00 (BV)	10	
Net community production (NCP) and community	Every 2 days	IWS	4:00 (BC)	2.5	Maugendre et al. (in press, this issue-b)
Gross primary production (¹⁸ O-GPP, BC only)	Every 4 days		5:00 (BV)	2.0	
Carbon fixation (¹⁴ C, BV only)	Every 2 days		0.5		
Heterotrophic production	Every 2 days	IWS	4:00 (BC)	0.5	Celussi et al. (in press, this issue)
Enzymatic activities			5:00 (BV)	0.5	
Viral production rates (BV only)	Every 4 days	IWS	5:00 (BV)	1	Tsiola et al. (sbm, this issue)
Copepod feeding (BC only)	On 4 occasions	S	8:30 (BC)	15	Zervoudaki et al. (sbm, this issue)
Copepod production (BC only)	Every 2 days	IWS	10:30 (BC)	5	
Carbon transfer					
¹³ C in dissolved inorganic carbon (¹³ C-DIC, water column)	Daily after addition then every 2 days	IWS	8:30	0.12	Maugendre et al. (in press, this issue-a)
¹³ C in particulate organic carbon (¹³ C-POC, water column)			8:30	1-1.5	
¹³ C in phospho-lipid fatty acids (¹³ C-PLFA, water column)			8:30	4	
¹³ C in particulate organic carbon (¹³ C-POC, sediment traps)	Daily (BC) Every 2 days (BV)	15:00	-		
¹³ C in zooplankton (¹³ C-zoo, water column, only BC)	End of experiment	WP2 Net	10:00	-	
Sedimentation					

Particulate organic carbon (POC)

Daily (BC)
Every 2 days (BV)

Diving

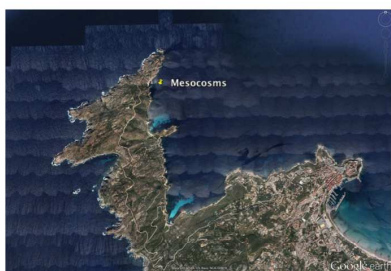
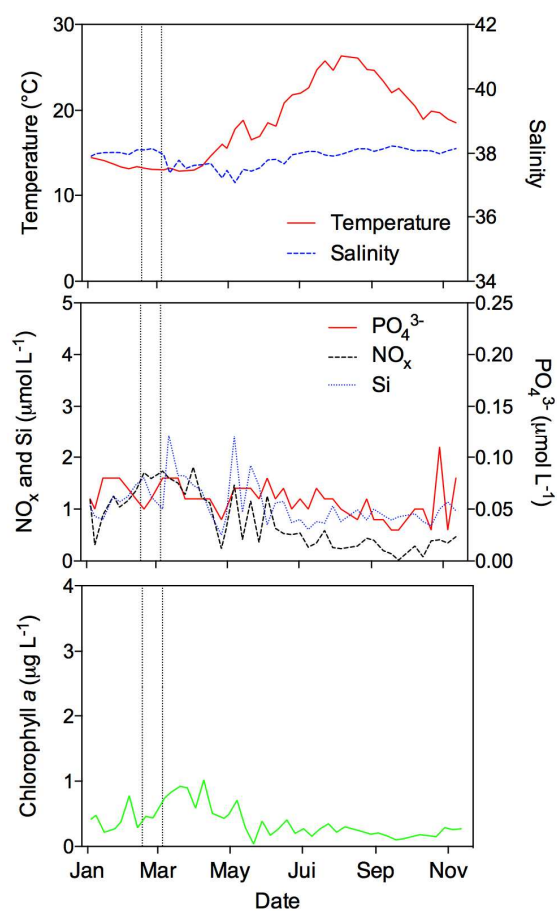
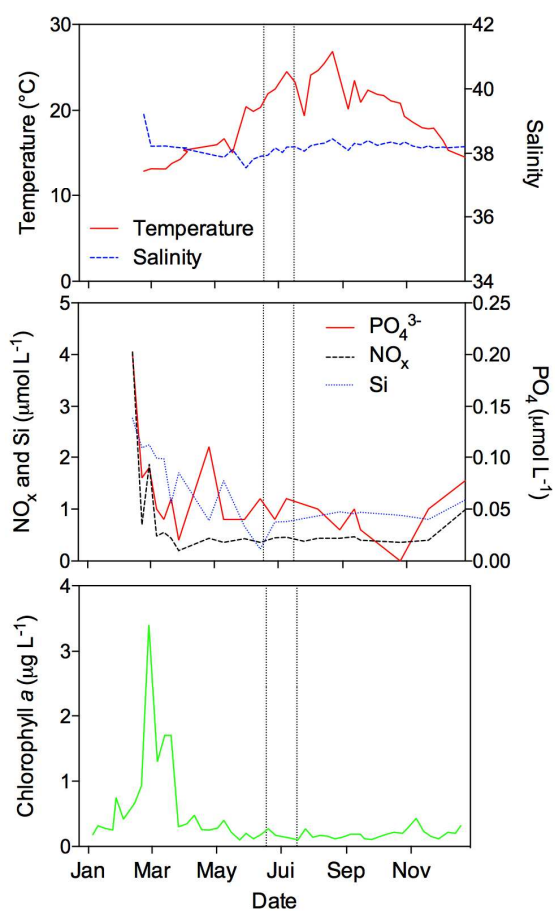
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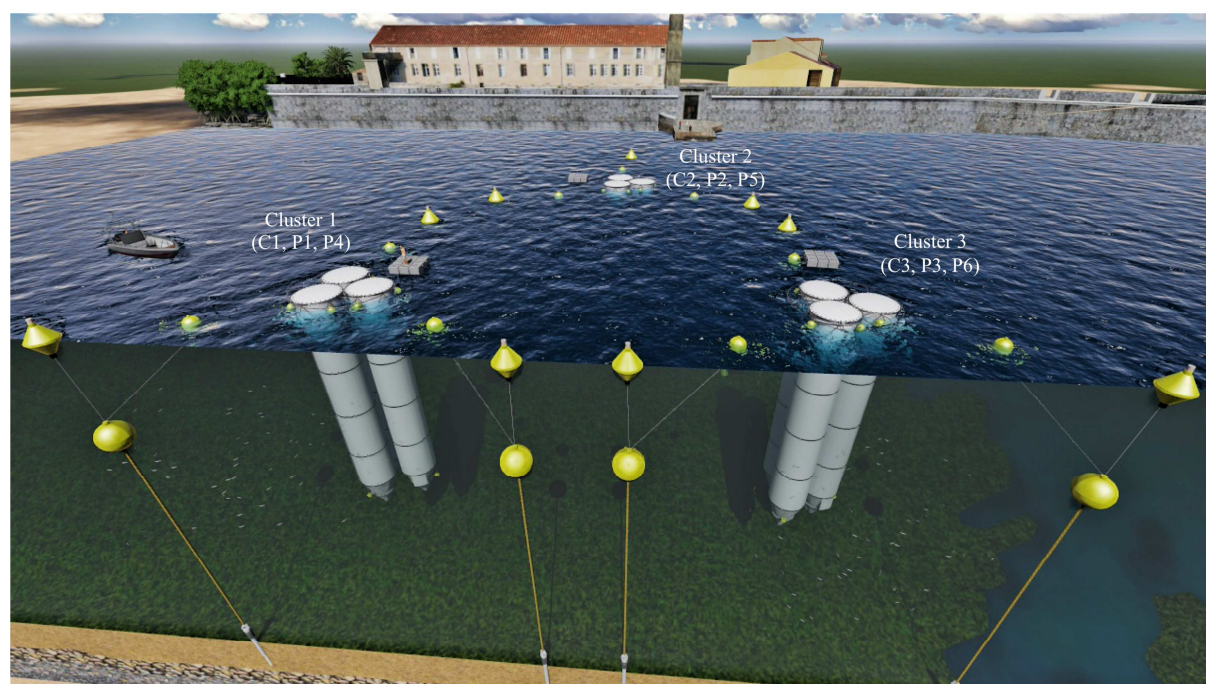
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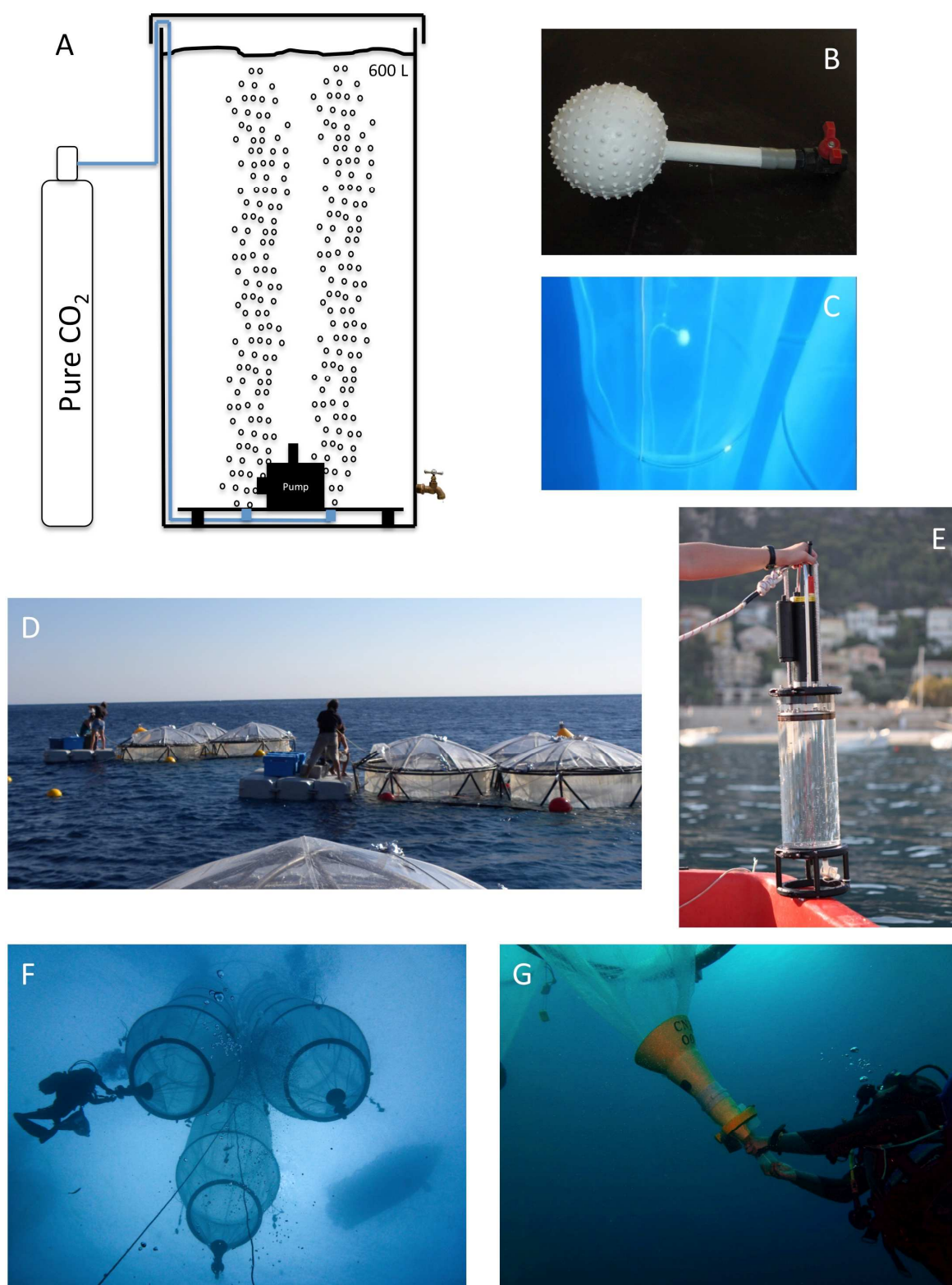
Gazeau et al. (sbm, this issue)

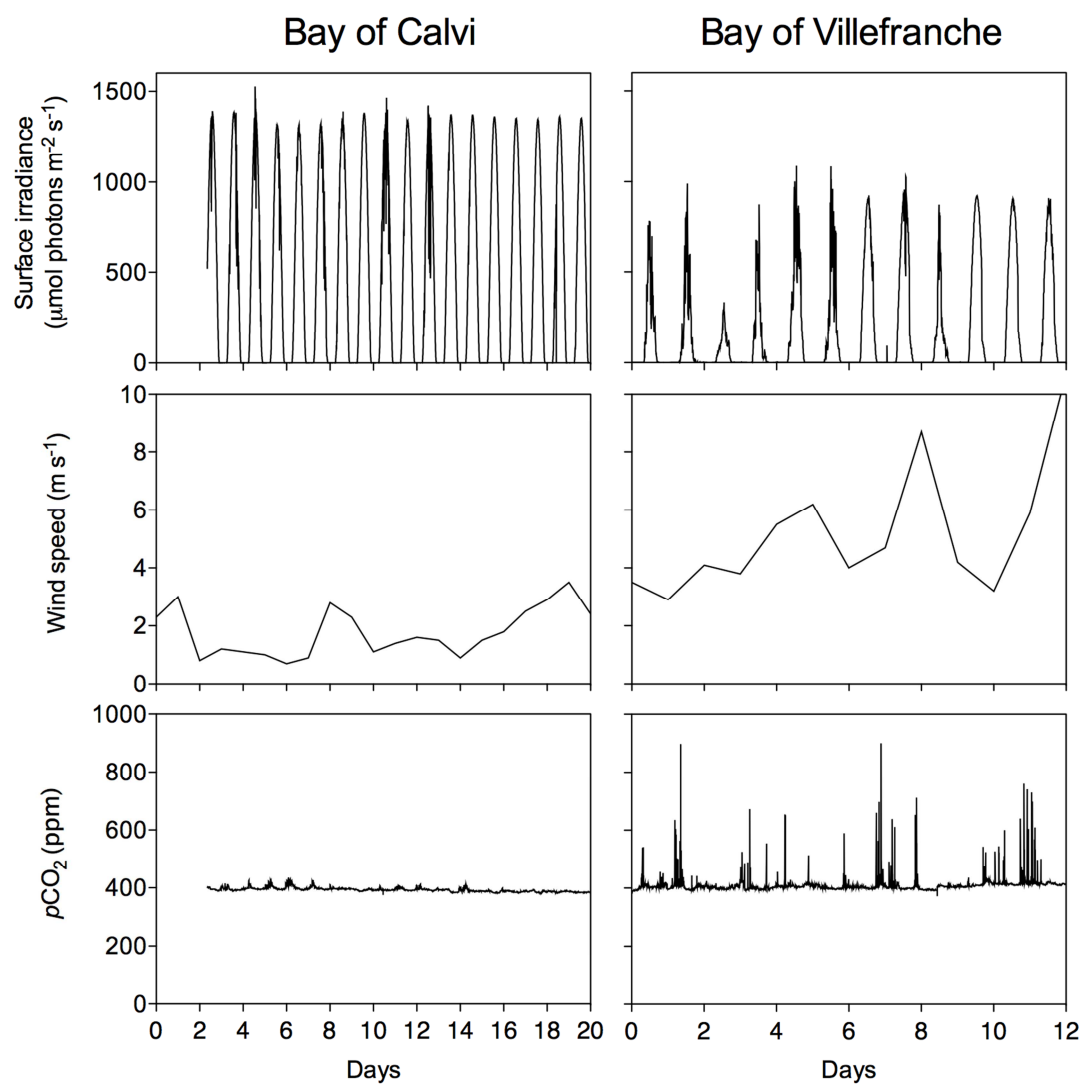
Table 5. Volumes (in L) of the 9 mesocosms estimated based on salt-addition on July 17th in the Bay of Calvi. Removal (sampling, evaporation) and addition of water (acidification) are also reported. Volume of P6 has been recalculated assuming an initial volume as equal to the mean of the 8 other mesocosms, see text of section 3.5 for details.

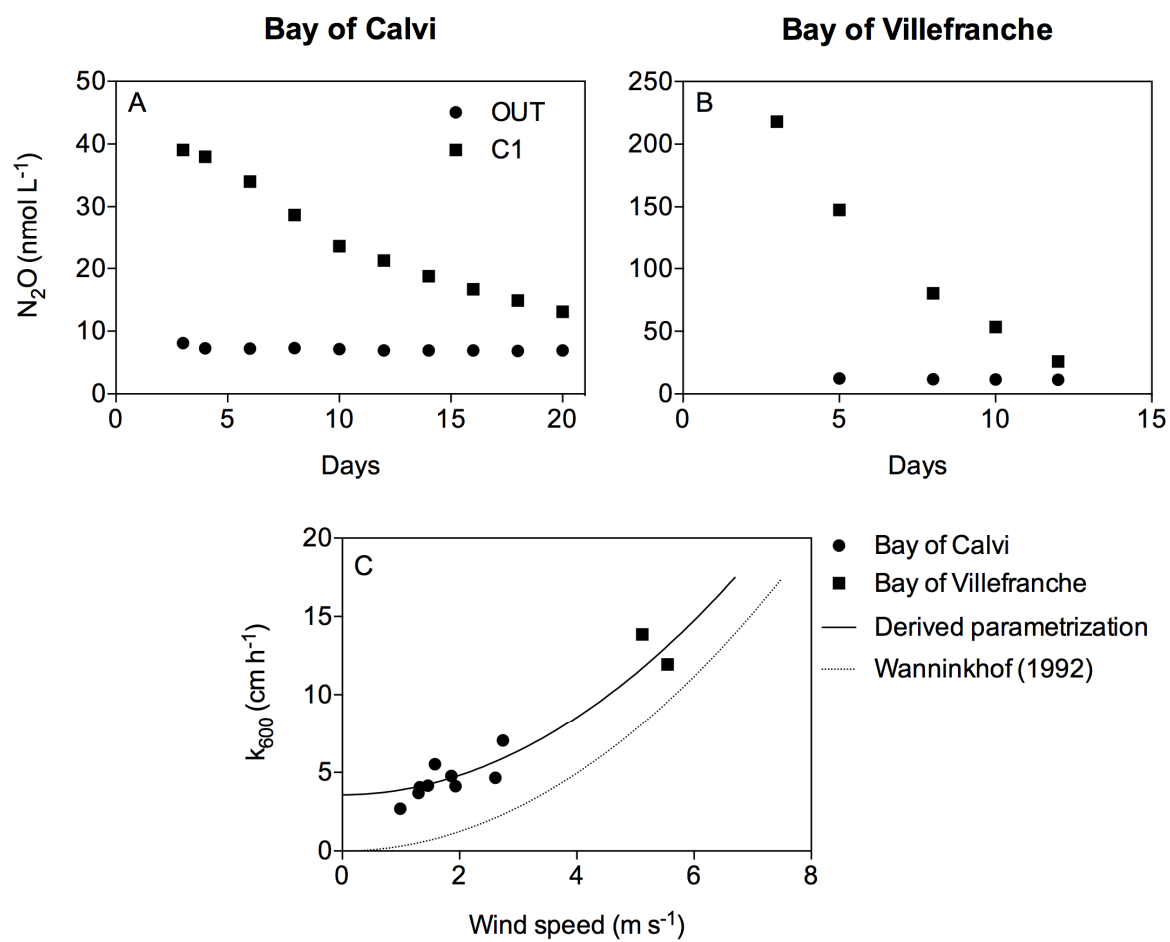
	Date		C1	C2	C3	P1	P2	P3	P4	P5	P6	P6 corrected
	19/06/2012	Initial volume	42141	44369	44540	46344	42962	44423	44874	43930	51596	44198
Acidification phase	20/06/2012	Acidification				+75	+150	+200	+225	+270	+330	
	-	Sampling	-20	-20	-20	-20	-20	-20	-20	-20	-20	
	23/06/2012	Evaporation	-38	-51	-37	-54	-60	-32	-52	-45	-68	
Experimental phase	24/06/2012	Sampling	-650	-668	-650	-668	-650	-668	-650	-650	-668	
	-	Evaporation	-125	-224	-154	-258	-250	-222	-232	-160	-241	
	14/07/2012											
	16/07/2012	Final volume (estimated)	41308	43423	43660	45437	42132	43746	44144	43325	50929	43531

**Bay of Calvi****Bay of Villefranche**

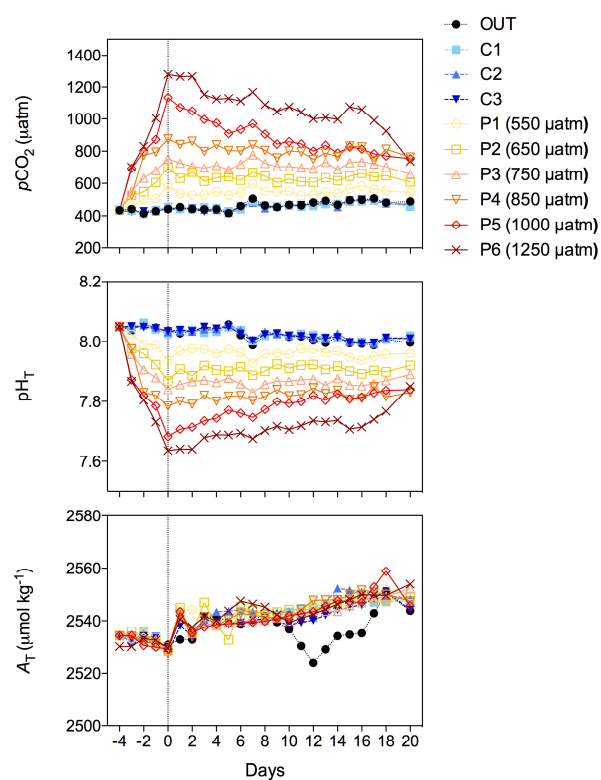




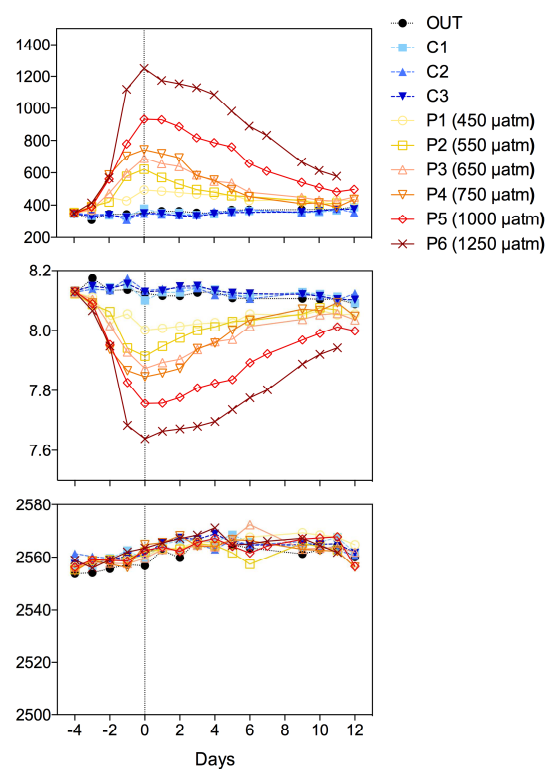


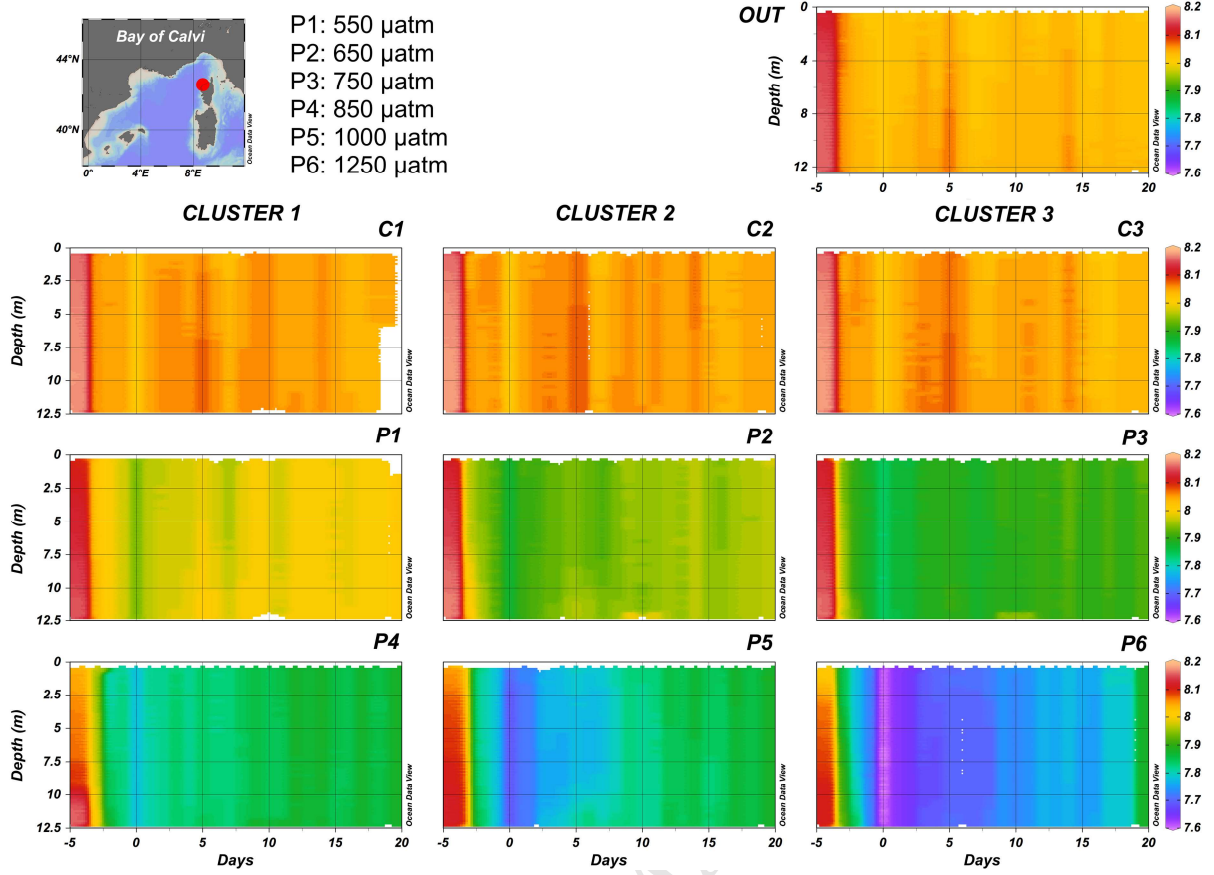


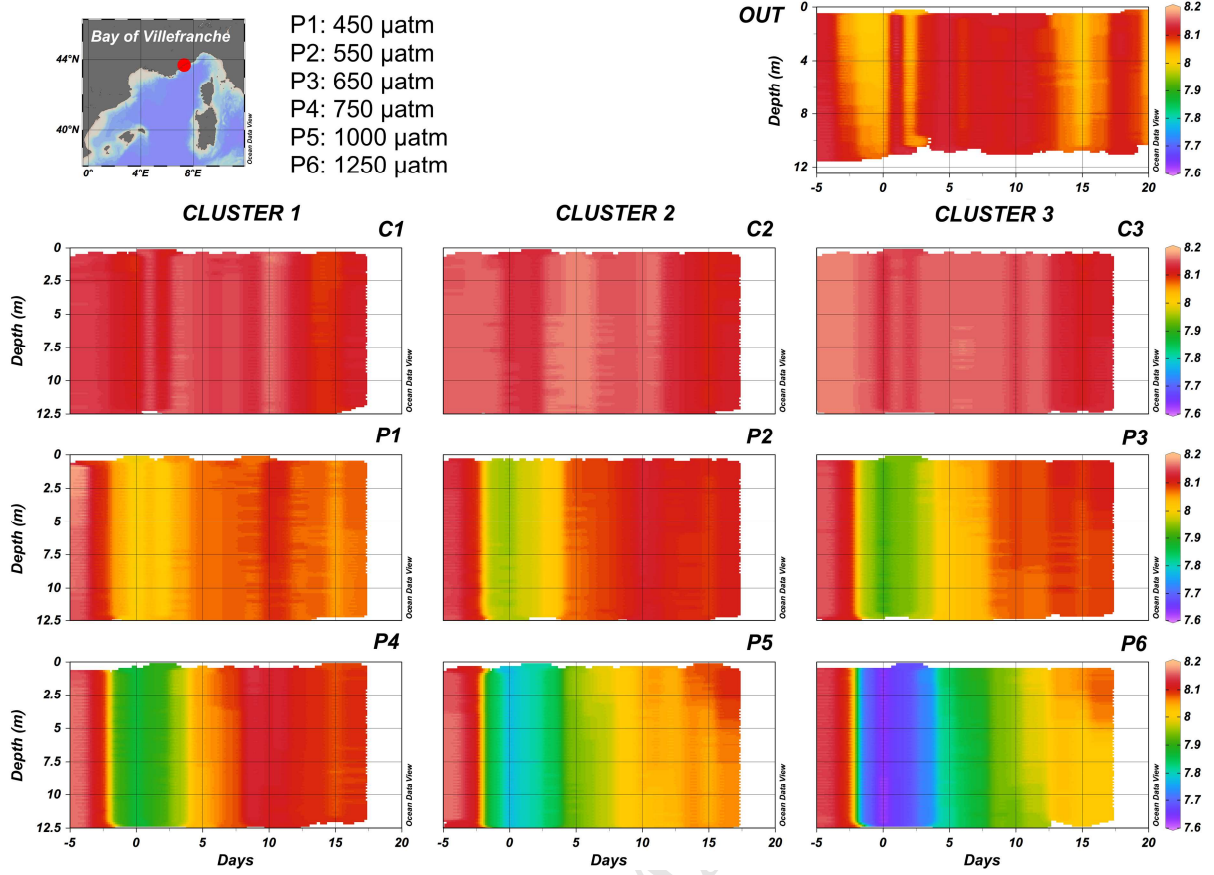
Bay of Calvi



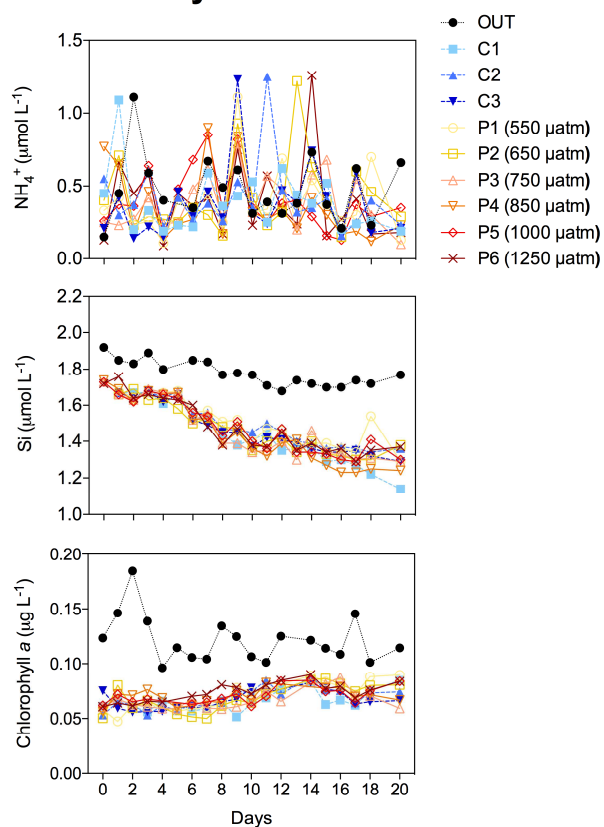
Bay of Villefranche







Bay of Calvi



Bay of Villefranche

