# Limited impact of ocean acidification on phytoplankton community structure and carbon export in an oligotrophic environment: Results from two short-term mesocosm studies in the Mediterranean Sea

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Highlights:

- Two large mesocosm experiments carried out in the Northwestern Mediterranean Sea
- Experiments conducted in the summer oligotrophic *vs.* winter mesotrophic periods
- Production limited by nutrient availability and community dominated by small species
- Organic matter export was not impacted by $CO_2$-enrichment
- In areas where nutrient availability exerts a strong pressure on phytoplankton growth, $CO_2$ addition will likely have very limited effects on phytoplankton diversity

Keywords:

Ocean acidification; Pelagic mesocosms; Mediterranean Sea; Oligotrophic area; Phytoplankton community

# Abstract

Modifications in the strength of the biological pump as a consequence of ocean acidification, whether positive or negative, have the potential to impact atmospheric $CO_2$ and therefore climate. So far, most plankton community perturbation studies have been performed in nutrient-rich areas although there are some indications that $CO_2$-dependent growth could differ in nutrient-replete vs. -limited regions and with different community compositions. Two *in situ* mesocosm experiments were performed in the NW Mediterranean Sea during two seasons with contrasted environmental conditions: summer oligotrophic stratified waters in the Bay of Calvi vs. winter mesotrophic well-mixed waters in the Bay of Villefranche. Nine mesocosms were deployed for 20 and 12 d, respectively, and subjected to seven $CO_2$ levels (3 controls, 6 elevated levels). Both phytoplankton assemblages were dominated by pico- and nano-phytoplankton cells. Although haptophyceae and dinoflagellates benefited from short-term $CO_2$ enrichment in summer, their response remained small with no consequences on organic matter export due to strong environmental constraints (nutrient availability). In winter, most of the plankton growth and associated nutrient consumption occurred during the 4-day acidification period (before the experimental phase). During the remaining experimental period, characterized by low nutrient availability, plankton growth was minimal and no clear $CO_2$-dependency was found for any of the tested parameters. While there is a strong confidence on the absence of significant effect of short-term $CO_2$ addition under oligotrophic conditions, more investigations are needed to assess the response of plankton communities in winter when vertical mixing and weather conditions are major factors controlling plankton dynamics.

# 1. Introduction

During the last 150 years, human activities, through the combustion of fossil fuels (oil, gas and coal), have led to a dramatic release of carbon dioxide ($CO_2$) to the Earth's atmosphere. The accumulation of $CO_2$ impacts the radiative forcing, thereby warming the atmosphere and the ocean. The ocean acts as a climate integrator that absorbed 93% of Earth's additional heat since the 1970s, offsetting much atmospheric warming but increasing ocean temperature and sea level and captured 28% of anthropogenic $CO_2$ emissions since 1750 (Gattuso et al., 2015). Although providing a valuable human service by moderating the rate and severity of global warming, the consequence of this oceanic $CO_2$ pump is the on-going increase in ocean acidity (i.e. decrease in pH). Surface ocean pH has already decreased by 0.1 units since the beginning of the industrial era (i.e. increased acidity of 30%; Ciais et al., 2013). According to recent projections and depending on the emission scenario considered, an additional decrease ranging between 0.06 and 0.32 units is expected by 2100 (Ciais et al., 2013).

The decrease in seawater pH leads to a decrease in the concentration of carbonate ions ($CO_3^{2-}$), one of the building blocks of calcium carbonate ($CaCO_3$), and alters the ability of many calcifying organisms to precipitate $CaCO_3$ (e.g. Kroeker et al., 2013). In addition, a decrease in seawater pH leads to an increase in dissolved $CO_2$ and bicarbonate ($HCO_3^-$) concentrations. Carbon fixation by marine photosynthetic organisms represents about 50% of global Earth primary production (Field et al., 1998), and the export of part of the produced organic matter from the sunlit surface layer to the deep-ocean (i.e. the biological or soft-tissue pump) is responsible for ~70% of surface to deep-ocean dissolved inorganic carbon ($C_T$) gradients (Sarmiento and Gruber, 2006). Therefore, modifications in the strength of this

biotically mediated carbon pump, whether positive or negative, have the potential to impact atmospheric $CO_2$ and therefore climate (Riebesell et al., 2007).

$CO_2$ rather than the much more abundant $HCO_3^-$ is the substrate used in the carbon fixation step of photosynthesis and RubisCO, the enzyme catalyzing this reaction, has a low affinity for $CO_2$ (Badger et al., 1998; Giordano et al., 2005). As such, this enzyme is theoretically not saturated under current ambient $CO_2$ levels (Badger et al., 1998). However, nearly all marine species have developed various mechanisms (carbon concentration mechanisms or CCMs) to compensate for this low $CO_2$ availability through the energy-demanding use of carbonic anhydrase enzymes or active $CO_2$ and/or bicarbonate transports through membranes (Raven et al., 2014). There is evidence that both the RubisCO affinity for $CO_2$ as well as the efficiency of these CCMs differ widely among taxa, species or even strains (Tortell, 2000; Young et al., 2016), complicating the prediction of whether a cell's carbon fixation rate will respond directly to ambient changes in $CO_2$ availability through increased $CO_2$ diffusion and/or less energy expenditure needed to operate CCMs (Mangan et al., 2016; Raven and Beardall, 2014). Finally, although downregulation of CCMs at elevated $CO_2$ has been observed, the significance of this downregulation to overall cell physiology and growth is not currently well constrained due to the presence of other limiting factors in the oceans such as macro- or micro-nutrients and light (Hennon et al., 2015; Young and Morel, 2015). All of this can partly explain the very diverse findings that have been documented on the effect of increased ambient $CO_2$ availability on photosynthesis and growth of marine phytoplankton (Dutkiewicz et al., 2015).

Apart from the above-mentioned variability in RubisCO affinity for $CO_2$ and CCMs efficiency, a significant part of the observed discrepancies among available perturbation studies could be explained by differences in experimental setups and environmental conditions such as temperature, light conditions and nutrient availability. Phytoplankton

growth obviously does not only depend on carbon availability but on a combination of physico-chemico-biological drivers such as macro- and micro-nutrient availability, temperature, light, competition and grazing. It is therefore very likely that the response of phytoplankton will differ depending on these environmental conditions (Verspagen et al., 2014). Furthermore, as this is the amount of organic matter that can escapes the sunlit layer that determines the capacity of the surface ocean to pump atmospheric $CO_2$, there is a great need to evaluate the impact of $CO_2$, not only on phytoplankton growth but on the export of this organic matter to deeper layers. The build-up of organic matter and its potential export strongly depends on phytoplankton community composition (Eggers et al., 2014). Indeed, large cells (e.g. diatoms) account for a large proportion of export production and ultimate burial in sediments (Finkel et al., 2005). In contrast, small cells (nano- and pico-plankton) are particularly important in regions with limited nutrient availability with a close coupling between production and grazing through the microbial loop and a with low export capacity (Riebesell and Tortell, 2011). As already mentioned, differing responses to increased $CO_2$ availability between different functional groups, size classes and species (Dutkiewicz et al., 2015) have the potential to significantly alter community structure and functioning. In that sense, studies focused on plankton assemblages rather than on isolated single species and under very contrasted environmental conditions are very informative (Tarling et al., 2016).

During the last decade, there has been a noticeable increase in the number of experimental assessments of the sensitivity of plankton community compositions to the on-going increase in $CO_2$. These experiments were conducted in various areas of the world ocean using different approaches, from small bottle incubations to large mesocosm deployments, and over different time scales (few days to few weeks). Several of these experiments highlighted significant modifications of community compositions under elevated $CO_2$ levels. For instance, $CO_2$ enrichment has been shown to stimulate growth of large species such as

diatoms (e.g. Domingues et al., 2014; Feng et al., 2009; Reul et al., 2014; Tortell et al., 2002; Tortell et al., 2008; Wu et al., 2014). Several experiments suggested stimulating effects on small species (pico-phytoplankton; e.g. Newbold et al., 2012; Paulino et al., 2008; Schulz et al., 2013). In contrast, Richier et al. (2014) reported significant decrease in the growth of small phytoplankton species (< 10 μm) suggesting that small species are less adapted to changes in their local pH while larger cells must face larger pH variations at short time scales (Flynn et al., 2012). Other studies showed differential responses between species from the same taxa (e.g. Endo et al., 2016; Feng et al., 2010; Kim et al., 2006; Meakin and Wyman, 2011) and finally among different phylotypes and phenotypes of the same species (e.g. Brading et al., 2011; Rickaby et al., 2016).

Whether or not these modifications of community structure (e.g. increase or decrease in cell size) can modify the amount of organic matter sinking to deeper layers can be evaluated through the use of mesocosms. They 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 and the collection of sinking organic matter (Riebesell et al., 2008; Riebesell et al., 2013a). In recent years, plankton community studies performed using such experimental systems have led to very contrasted outcomes in terms of community composition and carbon export responses to $CO_2$ enrichment (see Table 1). Most of these experiments have been performed in nutrient-rich areas (or following artificial nutrient enrichment) dominated by large species and experiments conducted in areas limited by nitrate, phosphate and/or iron are currently lacking (Paul et al., 2015a). These areas represent a very large surface area of the ocean and are projected to expand in the coming decades because of enhanced thermal stratification and nutrient depletion (Irwin and Oliver, 2009; Polovina et al., 2008). As already mentioned, they are usually dominated by small cells adapted to low-nutrient conditions and have low export capacities. Recently, and in contrast

to theoretical considerations (Verspagen et al., 2014), two mesocosm experiments suggested that communities exposed to low nutrient concentrations may be more responsive to $CO_2$ enrichment than previously thought (Bach et al., 2016; Paul et al., 2015a). This was confirmed recently by Sala et al. (2016) based on indoor experiments in a coastal site of the Western Mediterranean Sea. During these experiments, effects of ocean acidification, i.e. positive effect on pico- and nano-phytoplankton, were more important when nutrient concentrations were low. However, it must be stressed that nutrient and chlorophyll levels observed during these experiments were representative of an urbanized coastal area and much higher than levels usually observed in the vast majority of the Mediterranean Sea.

The Mediterranean Sea is generally considered as oligotrophic but actually exhibits a gradient from mesotrophic-oligotrophic in the western basin to ultra-oligotrophic in the eastern basin (The Mermex group, 2011). These features are induced by the different localizations of the physical (the winter mixed layer) and nutrient (the nutricline) vertical interfaces, which are both determined by the large-scale circulation pattern (The Mermex group, 2011). Based on satellite-derived estimates, chlorophyll *a* concentrations exhibit low values (less than 0.2 $\mu g\ L^{-1}$) over most of the Mediterranean Sea, except for the Liguro-Provençal region where large blooms can be observed in late winter-early spring (D'Ortenzio and d'Alcala, 2009). Overall, phytoplankton communities are dominated by pico-phytoplankton (Siokou-Frangou et al., 2010). However, because of its very diversified (spatially and temporally) physical structure, localized higher nutrient availability can drive more intense biological activities and transient dominance of larger species such as diatoms and dinoflagellates (Bustillos-Guzmán et al., 1995). Diatoms are more abundant during the transition between mixed and stratified conditions (Claustre et al., 1994). These features make the Mediterranean Sea a perfect natural laboratory to study the effects of nutrient availability and community composition on the response of plankton community to $CO_2$ enrichment.

In the frame of the European project 'Mediterranean Sea Acidification under changing climate' (MedSeA; http://medsea-project.eu), for the first time, two short-term *in situ* mesocosm experiments were performed in the Northwestern Mediterranean Sea during two seasons with contrasted environmental conditions (i.e. summer oligotrophic stratified waters vs. winter mesotrophic well-mixed waters) and different phytoplankton community compositions (i.e. higher proportion of diatoms and lower proportion of pico-phytoplankton and cyanophyceae in winter compared to summer). In this paper, we report on the response of the phytoplankton community composition as well as of particulate organic matter dynamics and export to $CO_2$-enrichment.

# 2. Material and Methods

## 2.1. Study sites and experimental set-up

Two mesocosm experiments were conducted in the Northwestern Mediterranean Sea: the first one, in the Bay of Calvi (Corsica, France) in summer (June-July 2012), and the second one in the Bay of Villefranche (France) in winter (February-March 2013). The experimental set-up and mesocosm characteristics are fully described in Gazeau et al. (in press, this issue). Briefly, for each experiment, nine mesocosms of ca. 50 $m^3$ (2.3 m in diameter and 12 m deep) were deployed for 20 and 12 days in the Bay of Calvi and the Bay of Villefranche, respectively. Once the bottom of the mesocosms was closed, $CO_2$ saturated seawater was added to obtain a $pCO_2$ gradient across mesocosms ranging from ambient levels to 1,250 µatm, with three control mesocosms (C1, C2 and C3) and six mesocosms with increasing $pCO_2$ (P1 to P6). In the Bay of Calvi, the six targeted elevated $pCO_2$ levels were P1: 550, P2: 650, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. In the Bay of Villefranche, the levels were P1: 450, P2: 550, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. Mesocosms were grouped in clusters of 3 with each cluster containing a control, a medium and a high

213 $pCO_2$ level (cluster 1: C1, P1, P4; cluster 2: C2, P2, P5 and cluster 3: C3, P3, P6).
214 Acidification of the mesocosms was performed over four days by homogenous addition of
215 various volumes of $CO_2$-saturated seawater. Once targeted $pCO_2$ levels were reached, the
216 experiment started (day 0 = 24 June 2012 and 22 February 2013 for the Bay of Calvi and the
217 Bay of Villefranche, respectively). No further $CO_2$ additions were performed during the
218 experiments and $pCO_2$ levels evolved in mesocosms as a consequence of air-sea fluxes,
219 temperature changes and plankton net community production. Weather permitting,
220 conductivity-temperature-depth (CTD) casts were performed on a daily basis in each
221 mesocosm and in the external environment. Surface irradiance (photosynthetically active
222 radiation; PAR) was measured continuously during the two experiments using a LI-COR LI-
223 192SA 2-Pi sensor connected to a LI-1400 data logger (Gazeau et al., in press, this issue).
224 Vertical attenuation coefficients were estimated daily in each mesocosms, based on PAR
225 profiles (0-12 m) performed using a QSP-2200 4-Pi sensor (Biospherical Instruments Inc.)
226 mounted on the CTD. Mean daily photon doses were calculated using surface PAR and
227 estimated attenuation coefficients. In the Bay of Calvi, wind speed and direction were
228 recorded with a THIES© anemometer deployed, by the University of Liège (Belgium), on top
229 of one of buildings of the at 11.8 m height at a distance of about 400 m from the mesocosms.
230 For the experiment in the Bay of Villefranche, wind speed data (daily averages) were
231 obtained from the Météo France station at the Nice-Côte d'Azur International Airport
232 (43°39'55" N, 7°12'48" E).

233 **2.2. Sampling and analytical methods**

234 Depth-integrated (0-10 m) samplings from the mesocosms and the external
235 environment (referred thereafter to as OUT) were performed daily at 8:30 (local time) during
236 both experiments. All three clusters were simultaneously sampled from a plastic platform by
237 three teams of two scientists, each using an integrating water sampler (IWS; HYDRO-

238 BIOS©). The IWS units were hanged on a Kevlar cordage and downcasts were performed
239 manually at a regular speed of 10 cm $s^{-1}$ after rinsing it outside the mesocosms.

240 Samples for pigment determination were taken every day at 8:30 (local time) during
241 both experiments. Two litres of sampled seawater were filtered onto GF/F. Filters were
242 directly frozen in liquid nitrogen and stored at -80 °C pending analysis at the Laboratoire
243 d'Océanographie de Villefranche (France). Filters were extracted at -20 °C in 3 mL methanol
244 (100%), disrupted by sonication and clarified one hour later by vacuum filtration through
245 GF/F filters. The extracts were rapidly analyzed (within 24 h) by high performance liquid
246 chromatography (HPLC) with a complete Agilent Technologies system. The pigments were
247 separated and quantified as described in Ras et al. (2008).

248 *Synechococcus*, *Prochlorococcus*, autotrophic pico-eukaryotes and nano-eukaryotes
249 abundances were determined by flow cytometry analysis from samples taken every 2 days at
250 4:00 and 5:00 in the Bay of Calvi and the Bay of Villefranche, respectively (local times).
251 Seawater samples (2 mL) from each mesocosm were immediately fixed with 0.2 µm pre-
252 filtered 25% glutaraldehyde (0.5% final concentration), kept at 4 °C for approximately 30
253 min, then flash frozen in liquid nitrogen and finally stored at -80 °C until further processing
254 (Troussellier et al., 1995; Vaulot et al., 1989). Single cell analysis was processed with a
255 maximum flow rate of 65 µL $min^{-1}$through a Becton Dickinson, FACSCalibur flow
256 cytometer, equipped with an air-cooled Argon laser emitting at 488 nm and analyzed with the
257 Cell Quest Pro software (Becton Dickinson). The sample volume analyzed per time unit was
258 accurately defined by systematically adding to the samples fluorescent latex beads
259 suspensions of 1 µm (Polysciences Inc., Europe) at a concentration of 2.5 x $10^5$ beads $mL^{-1}$.
260 The abundance of autotrophic prokaryotes and pico- and nano eukaryotes was assessed from
261 unstained samples following the method described by Marie et al. (1999). In the Bay of
262 Villefranche, four groups were determined based on the optical parameters characterizing

each cell. *Synechococcus* (< 1.5 µm) cells were detected by their signature in a plot of orange fluorescence (FL2, 565–592 nm wavelength ranges) vs. red fluorescence (FL3, > 620 nm). *Prochlorococcus*, autotrophic pico-eukaryotes (< 2 µm) and nano-eukaryotes (2 – 10 µm) were detected in a plot of SSC vs. red fluorescence (FL3, > 620 nm). In the Bay of Calvi, only abundances of *Synechococcus* spp. and autotrophic pico-eukaryotes were assessed.

For particulate element concentrations, sampled seawater (0.75 - 2 L) was filtered through pre-combusted glass-fiber filters. Particulate organic carbon (POC) and nitrogen (PON) were determined at the Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (Italy) using a CHNO-S elemental analyzer (Costech ECS4010) after acidification with 1 N HCl and high-temperature combustion.

Collection of sediment traps was performed by a diver on a daily basis in the Bay of Calvi and less regularly as a consequence of bad weather conditions in the Bay of Villefranche (see Gazeau et al., in press, this issue). 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, (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%). Swimmers (i.e. opportunistic copepods and other zooplankton species that swim into the traps; Lee et al., 1988) larger than 1 mm were removed (and discarded) and the remaining material was rinsed, centrifuged, freeze-dried and grinded. In the Bay of Calvi, as a consequence of low amounts of material especially at the end of the experiment, daily sediment traps samples were pooled as follows: days 5-7, 8-10, 11-14 and 15-19. Total particulate matter was weighed for flux determination and subsamples were used for POC and PON measurements performed on elemental

analyzers after acidification with 1N HCl. Samples from the experiment in the Bay of Calvi were analyzed at NIOZ-Yerseke (The Netherlands) on a Thermo Electron Flash 1112. Samples from the experiment in the Bay of Villefranche were analyzed at the Laboratoire d'Océanographie de Villefranche (LOV, France) on a Elementar Vario Pyrocube.

### 2.3. Data analysis and statistics

The contribution of each phytoplankton group to total phytoplankton biomass (chlorophyll *a*) was estimated by using the CHEMTAX program with input ratios from Rodriguez et al. (2006) and Not et al. (2007). These pigment ratios established for open ocean plankton communities were modified from the original values by comparing microscopic and flow cytometry counts to HPLC analyses from samples collected in the NW Iberian coast, an area dominated by pico- and nano-eukaryotes, as observed in our study.

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.

For these two experiments, we chose to follow a $CO_2$ gradient approach rather than to replicate certain levels (ANOVA approach). As already done in several similar perturbation experiments (Paul et al., 2015a; Riebesell et al., 2013b), stepwise multiple linear regression analyses were performed to establish relationships between environmental/experimental conditions including $pCO_2$ and (1) POC and PON fluxes to the sediment traps as well as their ratios, (2) water column POC and PON concentrations and their ratios as well as (3) chlorophyll *a*-equivalent biomass or abundances of the different identified groups. Besides $pCO_2$, other environmental conditions that have been considered were temperature, salinity, daily photon doses, daily averaged wind speeds and nutrient concentrations ($NO_x$: $NO_3^-$ + $NO_2^-$, ammonium: $NH_4^+$, phosphate: $PO_4^{3-}$, and, only for diatoms, silicate: Si). Integrated levels of temperature and salinity were acquired through the daily CTD casts performed in

each mesocosm. $NO_x$ and phosphate were measured using nanomolar techniques as described in Louis et al. (in press, this issue). Ammonium and silicate concentrations were determined as described by Gazeau et al. (in press, this issue). As fully described in Gazeau et al. (in press, this issue), daily $pCO_2$ levels in each mesocosm were determined from dissolved inorganic carbon, total alkalinity, temperature and salinity using the R package seacarb (Lavigne et al., 2014).

All analyses were performed using the R software (R Core Team, 2015) and were considered significant at a probability $p < 0.01$.

# 3. Results

## 3.1. Environmental and experimental conditions during both experiments

Conditions in each mesocosm at the start and at the end of both experiments (days 0 and 20 in the Bay of Calvi and days 0 and 12 in the Bay of Villefranche) are shown in Tables 2 and 3. For both experiments, $pCO_2$ values in $CO_2$-enriched mesocosms (P1 to P6) were close to targeted levels. Ambient $pCO_2$ levels were higher in the Bay of Calvi in summer as compared to the Bay of Villefranche in winter (~450 vs. 350 µatm respectively). While $pCO_2$ levels slightly decreased (pH levels slightly increased) in the Bay of Calvi during the course of the experiment, especially for high $CO_2$ mesocosms (P5 and P6), drops in $pCO_2$ levels (increases in pH levels) were much stronger in the Bay of Villefranche with mesocosms P1 to P4 showing very similar levels by the end of the experiment (Fig. 1). Hydrological data (temperature and salinity) are fully described in Gazeau et al. (in press, this issue). Briefly, while temperature levels in the Bay of Calvi gradually increased from ~22.1 °C on day 0 to ~24.2 °C on day 20, they were constant in the Bay of Villefranche at around ~13.2 °C. Salinity increased roughly by 0.1-0.2 units during both experiments because of evaporation. In winter in the Bay of Villefranche, surface irradiance was generally 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 the Bay of Villefranche, minimal and maximal daily (sunrise to sunset) average values of 103 and 513 µmol photons $m^{-2}$ $s^{-1}$ were recorded with a L:D regime of 11.5:12.5, and maximal irradiance levels (~300-1100 µmol photons $m^{-2}$ $s^{-1}$) reached at 1:00 pm. Light attenuation coefficients were generally constants during both experiments with higher values estimated in winter in the Bay of Villefranche than in summer in the bay of Calvi (0.19 ± 0.07 SD and 0.14 ± 0.05 SD $m^{-1}$,

346 respectively). Higher daily averaged wind speeds were recorded during the winter experiment
347 in the Bay of Villefranche with very windy conditions experienced on day 8 that prevented
348 sampling during that day, and even winder on day 12 and the following night that irreversibly
349 damaged the bags.

350 In summer in the Bay of Calvi, $NO_x$ concentrations initially decreased then increased
351 agin after day 14 to reach similar levels than at the start of the experiment (47 ± 20 on day 0
352 vs. 60 ± 15 nmol $L^{-1}$ on day 20; average ± SD between the nine mesocosms). Dissolved
353 inorganic phosphate ($PO_4^{3-}$) quickly decreased from day 0 to day 1 and remained constant
354 during the rest of the experiment (23 ± 12 on day 0 vs. 7 ± 2 nmol $L^{-1}$ on day 20). In winter in
355 the Bay of Villefranche, $PO_4^{3-}$ concentrations were generally similar to the ones encountered
356 in summer in the Bay of Calvi and no strong variations could be observed in all mesocosms
357 along the course of the experiment (global average: 9 ± 1 nM). $NO_x$ levels were much higher
358 in the Bay of Villefranche than in the Bay of Calvi when bags were closed (> 1 µM).
359 However, during the acidification phase (day-4 to -1), due to favorable weather conditions
360 (low wind, high irradiance levels, data not shown), chlorophyll *a* concentrations increased,
361 consuming a large proportion of available nitrate and nitrite before the start of the
362 experimental phase (day 0). As a consequence, while [$NO_x$] in external waters remained high
363 (~ 1 µM), all mesocosms were depleted in $NO_x$ with an average concentration of 129 ± 30
364 nM. $NO_x$ to phosphate ratios were higher in the Bay of Villefranche than in the Bay of Calvi
365 (2 ± 1 and 9 ± 4 on day 0 and on day 20 in the Bay of Calvi *vs.* 13 ± 4 and 33 ± 18 on day 0
366 and on day 12 in the Bay of Villefranche). More details on nutrient dynamics can be found in
367 Louis et al. (in press, this issue).

368 The experiment in the Bay of Calvi was representative of summer conditions in the
369 Ligurian Sea with low nutrient concentrations, low chlorophyll *a* concentrations (see below),
370 warm waters and high irradiance levels. In the Bay of Villefranche in winter, while

hydrological and weather conditions were typical of winter conditions in the Northwestern Mediterranean Sea (low temperature and irradiance levels), nutrients were rapidly depleted inside the mesocosms before the start of the experiment, and reached levels not usually encountered during this period of the year.

### 3.2. Phytoplankton assemblages during the summer experiment in the Bay of Calvi

Total chlorophyll *a* concentrations in the Bay of calvi (Fig. 2) averaged 0.07 ± 0.01 μg $L^{-1}$ in the nine mesocosms along the experiment, a value much lower than that in the surrounding waters (0.12 ± 0.02 μg $L^{-1}$). In mesocosms, chlorophyll *a* concentrations linearly increased during the experiment (GLM, $r^2 = 0.6$, $p < 0.001$) with a maximal concentration of 0.09 ± 0.003 μg $L^{-1}$ on day 14.

When pigment data for all mesocosms were pooled together (Fig. 3), the plankton community in the Bay of Calvi was found to be dominated at the start of the experiment by haptophyceae representing 36 ± 5% of the chlorophyll content, followed by cyanophyceae (20 ± 3%), chlorophyceae (14 ± 3%) and pelagophyceae (11 ± 2%). Important differences were identified along the experiment between concentrations of the different species inside the mesocosms and in the surrounding waters (Fig. 4). All species, except for diatoms, showed lower chlorophyll *a* biomass inside mesocosms. Diatoms were virtually absent in the surrounding waters, except at the end of the experiment. On day 20, while the contribution of cyanophyceae, dinophyceae, diatoms, pelagophyceae and cryptophyceae did not strongly change as compared to day 0, the contribution of chlorophyceae increased to 31 ± 4%.

Based on flow cytometry measurements, *Synechococcus* abundances increased during the first days of the experiment, reached maximal values on day 10 (averaged between mesocosms of 29600 ± 3000 cells $mL^{-1}$) and then decreased until the end of the experiment (Fig. 5). Similar dynamics, although with more variability among mesocosms, were observed

for autotrophic pico-eukaryotes, with abundances one order of magnitude lower than *Synechococcus*.

Table 4 shows total chlorophyll *a* concentrations were not correlated with $pCO_2$ but showed positive trends with salinity and to a lesser extent with $NH_4^+$. Chlorophyll *a*-equivalent biomass of two groups of phytoplankton were significantly correlated with $pCO_2$, dinophyceae and haptophyceae. For these two groups, $pCO_2$ appeared as the most important contributor to the variance. Note that a maximum of 66% of the variance (i.e. for chlorophyceae) observed in total chlorophyll *a* or group-specific biomasses could be explained by these stepwise linear regression analyses using the tested environmental and/or experimental variables.

### 3.3. Phytoplankton assemblage during the winter experiment in the Bay of Villefranche

In the Bay of Villefranche (Fig. 2), total chlorophyll *a* concentrations averaged 0.98 ± 0.15 μg $L^{-1}$ in the nine mesocosms along the 12-day experiment. Chlorophyll *a* remained slightly above levels in the surrounding waters for the entire experimental period, except for the last day (day 12) when concentrations increased abruptly outside the mesocosms. HPLC data are available for the acidification phase of this experiment (day -4 to day -1), data show that chlorophyll *a* concentrations increased during that period, consuming a large proportion of available nutrients, notably nitrate and nitrite, before the start of the experimental phase (see 3.1). In all mesocosms, after this initial peak, chlorophyll *a* concentrations linearly decreased until the end (GLM, $r^2 = 0.8$, $p < 0.001$).

When pigment data for all mesocosms were pooled together (Fig. 3), the plankton community in the Bay of Villefranche was dominated at the start of the experiment by cryptophyceae representing 26 ± 1% of the chlorophyll *a* content and by haptophyceae at the end (32 ± 5%). Following total chlorophyll *a* dynamics, almost all groups declined in terms of chlorophyll *a* equivalent biomass during the 12-day experiment except for cyanophyceae

420 whose biomass almost doubled between days 0 and 12 (Fig. 6). Groups that increased during
421 the acidification phase and consumed available nitrate and nitrite belonged to cryptophyceae,
422 haptophyceae, pelagophyceae and cyanophyceae. While pelagophyceae biomass remained
423 constant throughout the experiment, cryptophyceae biomass linearly declined and
424 haptophyceae showed maximal biomass on days 2 and 4 and then slightly declined. Several
425 groups did not follow the initial chlorophyll *a* increase during the acidification phase
426 (diatoms, dinophyceae, prasinophyceae and chlorophyceae).

427 Consistently with pigment data, flow cytometry data showed that *Synechococcus*
428 abundances significantly increased during the acidification phase and reached values much
429 above environmental (external) levels (Fig. 7). After few days of stagnation (days 0 to 6),
430 abundances further increased to maximal values on day 12 (averaged between mesocosms of
431 42600 ± 3000 cells $mL^{-1}$). In contrast, it appears that *Prochlorococcus* took less advantage of
432 this initial acidification phase with abundances on day 0 similar to external levels. After a
433 small initial decline, abundances increased during the entire experiment with increasing
434 variability between mesocosms. While autotrophic nano-eukaryotes abundance increased
435 before the start of the experiment to levels much higher on day 0 than in the surrounding
436 waters, no difference could be observed for autotrophic pico-eukaryotes on day 0 between
437 mesocosms and the surrounding waters. Autotrophic pico-eukaryote abundance decreased
438 until day 5, with very low variability between mesocosms, and increased until the end of the
439 experiment with much larger discrepancy between mesocosms. In contrast, abundances of
440 autotrophic nano-eukaryotes decreased almost linearly between day 0 and 12 with a large
441 inter-mesocosm variability throughout the experiment.

442 Table 4 shows that the chlorophyll *a*-equivalent biomass of haptophyceae and diatoms
443 were significantly correlated with $pCO_2$, although for none of these species $pCO_2$ appeared as
444 the most important contributor to the variance. While haptophyceae appeared negatively

correlated to $pCO_2$, diatoms were positively correlated to this variable. Note that for these two groups, less than half of the variance could be explained by these multiple regressions. For most of the tested variables, salinity appeared as the most important co-variable, being either positively or negatively correlated to them. As already mentioned, salinity increased gradually during the experiment and these correlations most likely reflect a time effect on these variables.

### 3.4. Particulate organic matter and export

In the Bay of Calvi, particulate C and N concentrations were very low and close to the analyzer detection limit (respectively, 2.9 - 7.4 μmol C $L^{-1}$ and 0.4 - 1.3 μmol N $L^{-1}$). C:N ratio of the particulate organic matter remained constant in the mesocosms throughout the experiment (7.0 ± 1.0) and very close to ambient conditions (7.1 ± 0.7; Fig. 2). In the Bay of Villefranche, higher POC an PON concentrations were measured (respectively, 7.9 - 20.2 μmol C $L^{-1}$ and 1.0 - 2.3 μmol N $L^{-1}$). As in the Bay of Calvi, C:N ratio of the particulate organic matter remained constant in the mesocosms throughout the experiment (7.5 ± 0.9) and lower than in the surrounding environment (12.9 ± 4.3; Fig. 2). During both experiments, none of the measured variables (POC, PON or POC:PON) displayed any observable dependence on seawater acidification (Table 4).

Particulate organic carbon and nitrogen export fluxes are presented in Fig. 8. During both experiments, although more visible in the Bay of Calvi, organic matter export rates were maximal at the start of the experiments and gradually decreased until the end of the experiments. Much more variability was observed in the Bay of Villefranche with higher exported quantities of organic matter. In the Bay of Calvi, exported C:N ratios were generally homogeneous between mesocosms at the start of the experiment and much more variability was observed towards the end. Stepwise linear regressions showed no $pCO_2$ effects on these export fluxes (Table 4).

# 4. Discussion

The overall objective of our study was to evaluate the response of the phytoplankton community, particulate organic matter dynamics and export to $pCO_2$ changes in the NW Mediterranean Sea under contrasted physico-chemical (e.g. hydrology, nutrients and irradiance) and biological conditions (assemblage composition and abundance). Unfortunately, both summer and winter experiments were conducted under nutrient limiting conditions on plankton communities dominated by small species.

The summer experiment in the Bay of Calvi was conducted under typical stratified summer conditions characterized by very low nutrient and chlorophyll concentrations and surface irradiance levels of ~1,400 µmol photons $m^{-2}$ $s^{-1}$, corresponding to maximal yearly values in that area (data not shown). With respect to nutrient availability, as fully discussed by (Louis et al., in press, this issue), observed $NO_x$ and phosphate depleted conditions during the experiment are in the range of usually observed values in the oligotrophic Mediterranean Sea in summer. At the start of the experiment, inorganic N:P ratio was 1.7 and increased up to ~4 in the mesocosms on day 20. Both a low N:P ratio and low nutrient concentrations suggest that this system experienced N and P co-limitation during this period (Louis et al., in press, this issue). During this experiment, the plankton community was clearly dominated by small phytoplankton cells such as haptophyceae, cyanobacteria and chlorophyceae. Similar conditions were reported in this area at this period of the year. Using the same mesocosm setup, Giovagnetti et al. (2013) showed that the summer plankton community was dominated by pico-phytoplankton, representing ~70% of total biomass and composed mostly of haptophyceae and cyanobacteria. The same experiment showed that nano- and micro-phytoplankton (~30% of total biomass) were composed of haptophyceae, chlorophyceae and dinoflagellates. During our experiment, phytoplankton biomass decreased during the

494 acidification phase in all mesocosms, independently of $pCO_2$ conditions, as shown by
495 fluorometric data acquired using daily CTD profiles (Gazeau et al., in press, this issue). This
496 corresponded to important organic matter sedimentation at the start of the experiment (first
497 few days) that further stabilized at low rates until the end of the experiment. No important
498 changes in the proportions of the different groups investigated were observed, at the exception
499 of chlorophyceae (see above)

500 At the end of our experiment and considering the averaged composition in all nine
501 mesocosms, dominance shifted towards chlorophyceae, in contrast to the external water
502 community which remained unchanged during the course of the experiment. This relative
503 overgrowth of chlorophyceae in all mesocosms, independently of $pCO_2$ conditions, was
504 potentially due to wall growth. Indeed, a strong wind and wave event prevented sampling on
505 day 19 (Gazeau et al., in press, this issue). On day 20 (our final sampling day), concentrations
506 in chlorophyceae (but also diatoms) increased significantly (+ 30%). This observed increase
507 was likely due to mesocosm shaking from wave actions, that released periphyton (i.e. species
508 growing on the wall of the mesocosms) in the water column. Large species such as diatoms
509 represented less than ~10% of phytoplankton biomass by the end of the experiment, although
510 biomasses were usually above those in external waters (~5 vs. < 1 ng $L^{-1}$). Obviously, this
511 does not appear as a surprise, as it is well known that, during the summer stratified period,
512 diatoms are outcompeted by small species, better adapted to low nutrient and high irradiance
513 levels, and usually do not represent more than 10% of the phytoplankton biomass in surface
514 waters of the Ligurian Sea (Navarro et al., 2014).

515 The winter experiment conducted in the Bay of Villefranche was carried out in order
516 to test for $CO_2$ enrichment effects on a Mediterranean plankton community not limited by
517 nutrient availability. However, as a consequence of very favorable weather conditions during
518 a short time window, much of the temporal dynamics observed during the experiment was

519 concentrated during the first few days before the end of the acidification process and nutrients
520 were rapidly consumed in the mesocosms. At the start of the experiment, when targeted $pCO_2$
521 levels were reached, most of the available $NO_x$ was already consumed and irradiance
522 conditions dropped significantly (Gazeau et al., in press, this issue) precluding the formation
523 of a real bloom in the bags. Addition of nutrients would have then been necessary to activate
524 plankton dynamics in the mesocosms but this strategy was not possible as we have been
525 forced to end the experiment after 12 days as a consequence of very bad weather conditions.
526 In the Bay, as a consequence of intense vertical mixing, chlorophyll concentrations have been
527 maintained at a lower level while nutrients have been continuously replenished (Louis et al.,
528 in press, this issue). In addition to probably not reflecting properly light conditions (Gazeau et
529 al., in press, this issue), the isolation of a water mass and the reduction of mixing certainly
530 does appear as a pitfall of this mesocosm approach or of any incubation system in these
531 ecosystems which dynamics is strongly linked to physico-chemical conditions (e.g. mixing,
532 irradiance). As such, results obtained during this experiment must be taken with extreme
533 caution because of conditions inside the mesocosms not fully reflecting winter conditions in
534 this area. Nevertheless, in winter in the Bay of Villefranche, phytoplankton biomass was
535 much higher than in summer in the Bay of Calvi with values around 1 μg $L^{-1}$ and a clear
536 dominance of small species such as haptophyceae, cryptophyceae and pelagophyceae (> 65%
537 of chlorophyll *a*-equivalent biomass). Previous observations at the entrance of the Bay of
538 Villefranche have shown that the spring phytoplankton bloom usually takes place in
539 February-March and is dominated by pico-nano-phytoplankton (Thyssen et al., 2014).
540 Although not always observed, this first bloom is followed by a second one in May that is
541 dominated by diatoms and large dinoflagellates (Bustillos-Guzmán et al., 1995; Gomez and
542 Gorsky, 2003). During this year 2013, the highest annual chlorophyll *a* concentrations were
543 reached later than observed in previous years (Gazeau et al., in press, this issue). Our

experiment therefore coincided with pre-bloom conditions although, again, nutrients were rapidly consumed in the mesocosms. Community composition did not drastically change during the course of the experiment inside the mesocosms but small phytoplankton species took advantage of the first few days of the experiment and are likely responsible for the strong consumption of $NO_x$ during the acidification phase (Louis et al., in press, this issue). In contrast, diatoms and dinoflagellates did not take advantage of the closing of the bags and of favorable weather conditions during these first few days and continuously decreased in abundance until the end of the experiment. This is not a surprise since these species are known to be outcompeted by smaller phytoplankton cells when nutrient limitation is temporally relieved after the winter vertical mixing (Bustillos-Guzmán et al., 1995). Instead of these species, autotrophic prokaryotes, especially *Synechococcus*, appeared to benefit from the closing of the bags, as their abundance was 3-fold higher than the ambient levels and kept increasing throughout the experiment. While autotrophic nano-eukaryotes decreased in abundance after the initial chlorophyll *a* increase during the acidification phase, autotrophic pico-eukaryotes benefited from the recycled nutrient pool, as a consequence of increasing bacterial abundance (Celussi et al., in press, this issue), and increased in number during the second part of the experiment.

During these two experiments, while total chlorophyll *a* concentrations appeared correlated to environmental conditions (e.g. nutrients, irradiance, salinity) and/or with time, no significant correlations were found with $pCO_2$. Similarly, we could not evidence any relationship between $pCO_2$ and POC or PON concentrations as well as organic carbon and nitrogen export to the sediment traps. When phytoplankton groups were analysed separately, positive effects were found for haptophyceae and autotrophic dinoflagellates in the Bay of Calvi during the oligotrophic summer period, similarly to what was found during a large *in situ* mesocosm experiment in the Arctic (Schulz et al., 2013). In winter in the Bay of

Villefranche, while haptophyceae were negatively correlated with $pCO_2$, diatoms appeared positively impacted, although for these two groups it must be stressed that $CO_2$ was not the first parameter driving their variance. Such positive $CO_2$-effects as observed in summer on haptophyceae and autotrophic dinoflagellates are not surprising, as these species do not possess very efficient CCMs (Reinfelder, 2011). Although cyanobacteria (including *Synechococcus*) appeared to benefit from our experimental conditions and from the very limited amount of nutrients, they were not impacted by $CO_2$-enrichment. These results are consistent to what was observed by Lomas et al. (2012) in the subtropical North Atlantic but stand in contrast to the negative impact of ocean acidification on *Synechococcus* abundance observed by Paulino et al. (2008) in a North Sea Fjord under very different trophic and experimental conditions compared to our experiments (i.e. higher chlorophyll levels as well as and enrichment with N and P). As suggested by Lomas et al. (2012), the response of cyanobacteria might be indirect and controlled by other variables such as nutrients.

All in all, the short-term addition of $CO_2$ in our nutrient-limited systems did not induce any clear effect on community composition based on pigment analysis. It must be stressed that these analyses do not allow detecting potential modifications/replacements at the specific or at the intra-specific level as suggested by several studies in the recent years (Brading et al., 2011; Rickaby et al., 2016). Nevertheless, scanning electron microscopy analyses reported by (Oviedo et al., in press, this issue) did not highlight any changes in coccolithophores and siliceous phytoplankton community compositions, and especially any changes in species size that could have an impact on sedimentation rates (Feng et al., 2010; Tortell et al., 2008; Wu et al., 2014). During our experiments, no phylogenetic studies have been conducted at the exception of diazotrophs during the summer in the Bay of Calvi (Rees et al., in press, this issue). For this group, no significant changes could be evidenced. In the present study, the small positive or negative effects that have been highlighted on selected

groups based on pigment analyses appear to be minimal and did not lead to significant changes in terms of community metabolism (Maugendre et al., in press, this issue-b), bacterial production (Celussi et al., in press, this issue), carbon transfer (Maugendre et al., in press, this issue-a) as well as carbon and nitrogen export (this study).

These results clearly stand in contrast to recent experiments conducted in a coastal site in the Western Mediterranean Sea, using indoor tanks (Sala et al., 2016). Similar to our planned experimental protocol, two experiments were conducted under contrasting conditions: winter, at the peak of the annual phytoplankton bloom, and summer, under low nutrient conditions. Their results suggested microbial communities will be considerably more affected by ocean acidification under oligotrophic conditions than in more productive waters. It must be stressed that even during their summer low-nutrient experiment, reported nitrate concentrations were almost ten times higher than concentrations observed in summer in the Bay of Calvi and four times higher than observed in the Bay of Villefranche in the mesocosms at the end of the acidification period. Similarly, chlorophyll concentrations during our summer experiment were three times lower than observed by Sala et al. (2016) in summer in the Bay of Blanes. Recently, two other experiments conducted using large *in situ* mesocosms also suggested that communities in nutrient-limited areas may be more responsive to changing carbonate chemistry than those having access to high inorganic nutrient concentrations (Bach et al., 2016; Paul et al., 2015a). These two experiments, sharing a similar experimental protocol than in the present study, were conducted over significantly longer time scales (> 43 days). During both experiments, impacts of elevated $CO_2$ were visible during the last phase when plankton communities were relying on remineralized nutrients. As both our experiments did not exceed ~20 days, the build-up of remineralized nutrients did not reach concentrations high enough to significantly relieve the nutrient limitation. Nutrient limitation can be episodically relieved in summer through atmospheric

inputs (The Mermex group, 2011 and references therein) and it is now well known that pulsed atmospheric nutrient inputs enhance phototrophic, heterotrophic and diazotrophic activities (Guieu et al., 2014). It appears therefore of the utmost importance to target future experimental efforts on the response of summer plankton communities to ocean acidification in the case of a transient relieve in nutrient limitation through of a dust deposition event.

# 5. Conclusion

To conclude, for the first time, short-term *in situ* pelagic mesocosm experiments have been conducted in LNLC areas of the Northwestern Mediterranean Sea to assess the response of phytoplankton communities to ocean acidification. In contrast to most previous mesocosm experiments, no nutrient addition took place during the experiments conducted in summer and winter. The summer plankton community was dominated by pico-phytoplankton and cyanobacteria and was strongly limited by $NO_x$ and phosphate availability. Although, haptophyceae and autotrophic dinoflagellates appeared to be favored by increased $CO_2$ availability during this short-term experiment, this benefit remained very minimal with no impact on carbon export, as a consequence of strong environmental constraints. The winter community was also dominated by small species (especially haptophyceae and cryptophyceae) that reacted soon after closing the bags and during the acidification period, possibly due to favorable weather conditions and irradiance levels. During this experiment, no signs of short term $CO_2$ addition dependency were detected on plankton community structure based on pigment analyses and on organic matter export. As a consequence of the very dynamic nature of environmental conditions and therefore of plankton biomass and composition in the Mediterranean Sea, more investigations are needed to carefully assess the response of plankton communities in winter when vertical mixing and weather conditions are major factors controlling plankton dynamics in this area. Future experimental protocols might

consider maintaining nutrient and chlorophyll levels as close as possible to ambient conditions over longer time scales. Although this might be experimentally challenging, we believe this is the only way to investigate these very dynamic communities. Finally, as atmospheric depositions in summer have the capacity to relieve nutrient limitations and to enhance plankton productions, there is a great need to perform future experiments considering these pulsed nutrient additions.

# Acknowledgements

This work was funded by the EC FP7 project 'Mediterranean Sea Acidification in a changing climate' (MedSeA; grant agreement 265103), the project 'European Free Ocean Carbon Enrichment' (eFOCE; BNP-Paribas Foundation), the MISTRALS-MERMEX program (Institut des Sciences de l'Univers, INSU), the Corsican local authorities and the Rhone-Mediterranean and Corsica Water Agency (http://www.eaurmc.fr). It is a contribution to the Surface Ocean-Lower Atmosphere Study (SOLAS) and Integrated Marine Biogeochemistry and Ecosystem Research (IMBER) projects. The STARESO marine station in Corsica is gratefully acknowledged for its assistance and boat support carried out within the framework of the STARECAPMED project funded by the Rhone-Mediterranean and Corsica Water Agency. B. Cataletto, F. Cerino, C. Comici, W. Dellisanti and C. Fabbro are acknowledged for sample preparation and analyses of particulate organic carbon and nitrogen. The staff of the Observatoire Océanologique de Villefranche is gratefully acknowledged for their assistance and boat support, colleagues of the Laboratoire d'Océanographie de Villefranche for providing laboratory space. S. Alliouane, B. Hesse, D. Luquet, D. Robin, P. Mahacek and E. Cox are acknowledged for assistance with diving operations. Thanks are due to all the MedSeA mesocosm team for help during the experiments. Finally, C. Brunet is acknowledged for his help on CHEMTAX analyses.

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# Figure legends

Figure 1. 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 $CO_2$ ($pCO_2$, upper panels) and pH on the total scale ($pH_T$, lower panels) were calculated using seacarb, based on dissolved inorganic carbon concentrations ($C_T$, not shown) and total alkalinity ($A_T$, not shown), measured daily from depth-integrated (0-10 m) samples. Vertical dotted lines show the start of the respective experiments (day 0).

Figure 2. Depth-integrated (0-10 m) chlorophyll *a* concentrations as measured by high performance liquid chromatography (HPLC; upper panels) and particulate organic carbon (POC) to particulate organic nitrogen (PON) ratio (POC:PON; lower panels) in the nine mesocosms and in the external environment during the experiment in the Bay of Calvi in summer 2012 (left panels) and in the Bay of Villefranche in winter 2013 (right panels). Vertical dotted lines on the right panels show the start of the experiment (day 0). No chlorophyll *a* and POC data are available before day 0 in the Bay of Calvi.

Figure 3. Averaged contribution (%) between all nine mesocosms of the main phytoplanktonic taxonomic groups to total chlorophyll *a* concentrations at the start (day 0) and at the end (day 20 or 12) of the experiments in the Bay of Calvi in summer 2012 (left panel) and in the Bay of Villefranche in winter 2013 (right panel).

Figure 4. Temporal evolution of chlorophyll *a* (chl *a*) -equivalent concentrations of eight taxonomic groups of phytoplankton during the experiment in the Bay of Calvi in summer 2012, in the nine mesocosms and in the external environment (OUT). Prasino: prasinophyceae, Dino: dinophyceae, Crypto: cryptophyceae, Hapto: haptophyceae, Pelago: pelagophyceae, Chloro: chlorophyceae, Cyano: cyanophyceae.

Figure 5. Temporal evolution of *Synechococcus* and pico-eukaryotes abundances as measured by flow cytometry, during the experiment in the Bay of Calvi in summer 2012, in the nine mesocosms.

Figure 6. Temporal evolution of chlorophyll *a* (chl *a*) -equivalent concentrations of 8 taxonomic groups of phytoplankton determined from high performance liquid chromatography (HPLC) measurements using modified CHEMTAX, during the experiment in the Bay of Villefranche in winter 2013, in the 9 mesocosms and in the external environment (OUT). Prasino: prasinophyceae, Dino: dinophyceae, Crypto: cryptophyceae, Hapto: haptophyceae, Pelago: pelagophyceae, Chloro: chlorophyceae, Cyano: cyanophyceae.

Figure 7. Temporal evolution of *Synechococcus*, *Prochlorococcus*, pico-eukaryotes and nano-eukaryotes abundances as measured by flow cytometry, during the experiment in the Bay of Villefranche in winter 2013, in the nine mesocosms and in the external environment (OUT).

Figure 8. Upper panel: temporal evolution of particulate organic carbon (POC) fluxes to the sediment traps during the experiment in the Bay of Calvi in summer 2012 (left panel) and in the Bay of Villefranche in winter 2013 (right panel). Lower panel: particulate organic carbon (POC) to particulate organic nitrogen (PON) ratio in the sediment traps during the experiment in the Bay of Calvi in summer 2012. No PON data available during the experiment in the Bay of Villefranche in winter 2013. Vertical dotted lines show the start of the respective experiment (day 0).

995 Table 1. Summary of past mesocosm (volume between 1 and 1000 $m^3$) ocean acidification experiments results on phytoplankton communities. ⇔, ⇧ and
996 ⇩ refer to neutral, positive and negative effects on chlorophyll *a* concentrations (Chl *a*) as well as concentrations of diatoms (diat), dinophyceae (Dino),
997 nano-eukaryotes (Nano), pico-eukaryotes (Pico) and cyanophyceae (Cyano). Impacts on carbon export are also reported when available. "√" indicates that
998 mesocosms were enriched with nutrients (Nut: nitrate, phosphate and sometimes silicate). "-" indicates that no information is available. Cryp and Chlo
999 refer to cryptophyceae and chlorophyceae respectively.

| Reference | Study location | Season | Nut | Major group | Chl | Diat | Dino | Nano | Pico | Cyano | Export | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Indoor** | | | | | | | | | | | | |
| Sommer et al. (2015)<br>Paul et al. (2015b) | Kiel Bight | Fall | | Diat/Dino | ⇔ | ⇩ | ⇔ | ⇔ | ⇔ | ⇔ | - | |
| **Outdoor - Floating raft** | | | | | | | | | | | | |
| Engel et al. (2005) | Norwegian Fjord | Spring | √ | Pico/Cyano | ⇔ | - | - | ⇔ | ⇔ | ⇔ | - | Decrease of coccolithophore calcification |
| Kim et al. (2006) | Korean coast | Fall | | Micro/Diat | - | ⇔ | - | - | - | - | - | |
| Engel et al. (2008) | Norwegian Fjord | Spring | √ | Pico | ⇔ | ⇔ | - | ⇩ | ⇧ | - | - | |
| Paulino et al. (2008)<br>Schulz et al. (2008) | Norwegian Fjord | Spring | √ | - | ⇔ | ⇔ | ⇔ | ⇔ | ⇧ | ⇩ | - | |
| Hopkins et al. (2010)<br>Meakin and Wyman (2011)<br>Newbold et al. (2012) | Norwegian Fjord | Spring | √ | Micro | ⇩ | - | - | ⇧ | ⇧ | ⇧ | - | Increase in large pico-eukaryotes |
| Kim et al. (2010)<br>Kim et al. (2011)<br>Kim et al. (2013) | Korean coast | Fall | √ | Diat/Dino | ⇔ | ⇧ | ⇔ | ⇧ | ⇔ | - | - | Shift from weakly to heavily silified diatoms |
| Calbet et al. (2014) | Norwegian Fjord | Spring | √ | Nano | ⇔ | ⇧ | ⇩ | ⇧ | ⇧ | - | - | |
| **Outdoor - Free floating** | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Schulz et al. (2013) | | | | Nano | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | Phase 1 before nutrient enrichment |
| Brussaard et al. (2013) | Arctic Fjord | Spring | √ | - | ⇧ | ⇔ | ⇧ | ⇧ | ⇧ | ⇔ | ⇔ | Phase 2 |
| Czerny et al. (2013) | | | | - | ⇩ | ⇔ | ⇧ | ⇔ | ⇧ | ⇔ | ⇩ | Phase 3 |
| Paul et al. (2015a) | Baltic Sea | Spring | | Cryp | ⇔ | ⇔ | - | ⇔ | ⇧ | ⇔ | ⇔ | Phase 1 |
| | | | | Chlo | ⇧ | ⇔ | - | ⇔ | ⇧ | ⇔ | ⇔ | Phase 2 |
| | | | | Chlo | ⇧ | ⇩ | - | ⇩ | ⇧ | ⇔ | ⇔ | Phase 3 |
| Bach et al. (2016) | Swedish Fjord | Winter | | - | - | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | - | |
| | | Spring | | Diatoms | - | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | - | First chlorophyll build-up |
| | | Spring | | Diatoms | - | ⇔ | ⇔ | ⇔ | ⇧ | ⇔ | - | Second chlorophyll build-up on remineralized nutrients |
| | | Spring | | - | - | ⇔ | ⇔ | ⇔ | ⇔ | ⇔ | - | |

1000 Table 2. Environmental and experimental conditions in the nine mesocosms and in the external environment (OUT) during the experiment in the
1001 Bay of Calvi in summer 2012. Levels of temperature (T in °C), salinity (S), partial pressure of $CO_2$ ($pCO_2$ in µatm), nitrate + nitrite ($NO_x$ in nmol
1002 $L^{-1}$) and phosphate ($PO_4^{3-}$ in nmol $L^{-1}$), ammonium ($NH_4^+$ in nmol $L^{-1}$) and silicate (Si in µmol $L^{-1}$) at the end of the acidification period (day 0)
1003 and at the end of the experiment (day 20) are reported. $NO_3^-$ and $PO_4^{3-}$ data from Louis et al. (in press, this issue). $NH_4^+$ and Si data are from
1004 Gazeau et al. (in press, this issue). NA: not available.

| | Day 0 | | | | | | | Day 20 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | S | $pCO_2$ | $NO_x$ | $PO_4^{3-}$ | $NH_4^+$ | Si | T | S | $pCO_2$ | $NO_x$ | $PO_4^{3-}$ | $NH_4^+$ | Si |
| OUT | 22.2 | 38.0 | 442 | 50 | 35 | 150 | 1.9 | 24.3 | 38.2 | 489 | NA | NA | 660 | 1.8 |
| C1 | 22.1 | 38.0 | 455 | 59 | NA | 450 | NA | NA | NA | 456 | 77 | 4 | 190 | 1.1 |
| C2 | 22.1 | 38.0 | 447 | 53 | 25 | 550 | NA | 24.2 | 38.2 | 472 | 61 | 6 | 230 | 1.4 |
| C3 | 22.1 | 38.0 | 444 | 69 | 21 | 210 | NA | 24.2 | 38.1 | 473 | 59 | 7 | 210 | 1.3 |
| P1 | 22.2 | 38.0 | 583 | NA | NA | 330 | NA | 24.3 | 38.2 | 544 | 45 | 6 | 130 | 1.3 |
| P2 | 22.1 | 38.0 | 698 | 37 | 23 | 400 | NA | 24.3 | 38.2 | 609 | 41 | 4 | 290 | 1.4 |
| P3 | 22.1 | 38.0 | 753 | 36 | 20 | 225 | 1.7 | 24.2 | 38.2 | 655 | 42 | 10 | 100 | 1.3 |
| P4 | 22.1 | 38.0 | 875 | 30 | 19 | 770 | 1.7 | 24.3 | 38.2 | 764 | 75 | 8 | 230 | 1.2 |
| P5 | 22.1 | 38.0 | 1134 | 37 | 31 | 260 | 1.7 | 24.3 | 38.1 | 754 | 76 | 9 | 350 | 1.3 |
| P6 | 22.1 | 38.0 | 1279 | 57 | NA | 130 | 1.7 | 24.2 | 38.2 | 738 | 61 | 8 | 180 | 1.4 |

Table 3. Environmental and experimental conditions in the nine mesocosms and in the external environment (OUT) during the experiment in the Bay of Villefranche in winter 2013. Levels of temperature (T in °C), salinity (S), partial pressure of $CO_2$ ($pCO_2$ in µatm), nitrate ($NO_3^-$ in nmol $L^{-1}$), phosphate ($PO_4^{3-}$ in nmol $L^{-1}$), ammonium ($NH_4^+$ in nmol $L^{-1}$) and silicate (Si in µmol $L^{-1}$) at the end of the acidification period (day 0) and at the end of the experiment (day 12) are reported. *No data are available for day 12 therefore levels on day 11 are reported. $NO_3^-$ and $PO_4^{3-}$ data from Louis et al. (in press, this issue). $NH_4^+$ and Si data are from Gazeau et al. (in press, this issue). NA: not available.

| | Day 0 | | | | | | | Day 12 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | S | $pCO_2$ | $NO_3^-$ | $PO_4^{3-}$ | $NH_4^+$ | Si | T | S | $pCO_2$ | $NO_3^-$ | $PO_4^{3-}$ | $NH_4^+$ | Si |
| OUT | 13.2 | 38.1 | 354 | 1166 | 10 | 62 | 1.3 | 13.2 | 38.2 | 391 | 1307 | 12 | 40 | 1.2 |
| C1 | 13.2 | 38.1 | 378 | 167 | 10 | 79 | NA | 13.2 | 38.2 | 388 | 394 | 9 | 49 | 1.0 |
| C2 | 13.2 | 38.1 | 347 | 118 | 12 | 57 | 1.1 | 13.2 | 38.2 | 354 | 194 | 11 | 31 | 1.1 |
| C3 | 13.2 | 38.1 | 350 | 110 | 9 | 81 | 1.2 | NA | NA | 376 | 127 | 10 | 26 | 1.2 |
| P1 | 13.2 | 38.1 | 494 | 135 | 10 | 73 | NA | 13.2 | 38.2 | 429 | 491 | 10 | 68 | 1.1 |
| P2 | 13.2 | 38.1 | 622 | 133 | 9 | 64 | 1.2 | 13.2 | 38.2 | 413* | NA | 10 | NA | NA |
| P3 | 13.2 | 38.1 | 691 | NA | 9 | 64 | 1.2 | NA | NA | 451 | 236 | NA | 26 | 1.2 |
| P4 | 13.2 | 38.1 | 744 | 72 | 12 | 80 | NA | 13.2 | 38.2 | 436 | 491 | 9 | 36 | 0.9 |
| P5 | 13.2 | 38.1 | 932 | 134 | 15 | 60 | 1.2 | 13.2 | 38.2 | 497 | 226 | 10 | 30 | 1.1 |
| P6 | 13.2 | 38.1 | 1250 | 156 | 8 | 60 | 1.1 | NA | NA | 579* | NA | NA | NA | NA |

Table 4. Stepwise multiple regression analysis between environmental/experimental variables (T: temperature, S: salinity, $pCO_2$: partial pressure of $CO_2$, $NO_3^-$: nitrate concentrations, $PO_4^{3-}$: phosphate concentrations, $NH_4^+$: ammonium concentrations, Si: silicate concentrations (only for diatoms), I: daily integrated photon doses and w: daily averaged wind speeds) and total chlorophyll *a* (chl *a*) concentrations or chlorophyll *a*-equivalent concentrations of eight taxonomic groups of phytoplankton determined from high performance liquid chromatography (HPLC) measurements using modified CHEMTAX or *Synechococcus*, *Prochlorococcus*, pico-eukaryotes and nano-eukaryotes abundances as measured by flow cytometry, during the experiment in the Bay of Calvi in summer 2012 and in the Bay of Villefranche in winter 2013. Note that *Prochlorococcus* and nano-eukaryotes abundances are not available for the Bay of Calvi and that PON fluxes are not available for the Bay of Villefranche (denoted as NA). Bold text denotes significant correlations ($p < 0.01$) between the considered variable and $pCO_2$ and the sign (+ or -) refers to the sign of the relationship between the considered variable and the environmental/experimental parameters considered. NS: not significant.

| Bay of Calvi | F | Adj. $r^2$ | df | Overall *p* | Variable | *Sign* | *p* |
|---|---|---|---|---|---|---|---|
| Particulate matter | | | | | | | |
| POC | NS | | | | | | |
| PON | 7.0 | 0.06 | 177 | | I | - | < 0.001 |
| | | | | | S | - | 0.003 |
| POC:PON | 5.2 | 0.09 | 174 | < 0.001 | T | + | < 0.001 |
| Pigments | | | | | | | |

| Bay of Villefranche | F | Adj. $r^2$ | df | Overall *p* | Variable | *Sign* | *p* |
|---|---|---|---|---|---|---|---|
| Particulate matter | | | | | | | |
| POC | 34.5 | 0.57 | 97 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| | | | | | S | - | < 0.001 |
| PON | 17.3 | 0.32 | 101 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| POC:PON | 9.5 | 0.14 | 99 | < 0.001 | $NH_4^+$ | + | 0.006 |
| Pigments | | | | | | | |

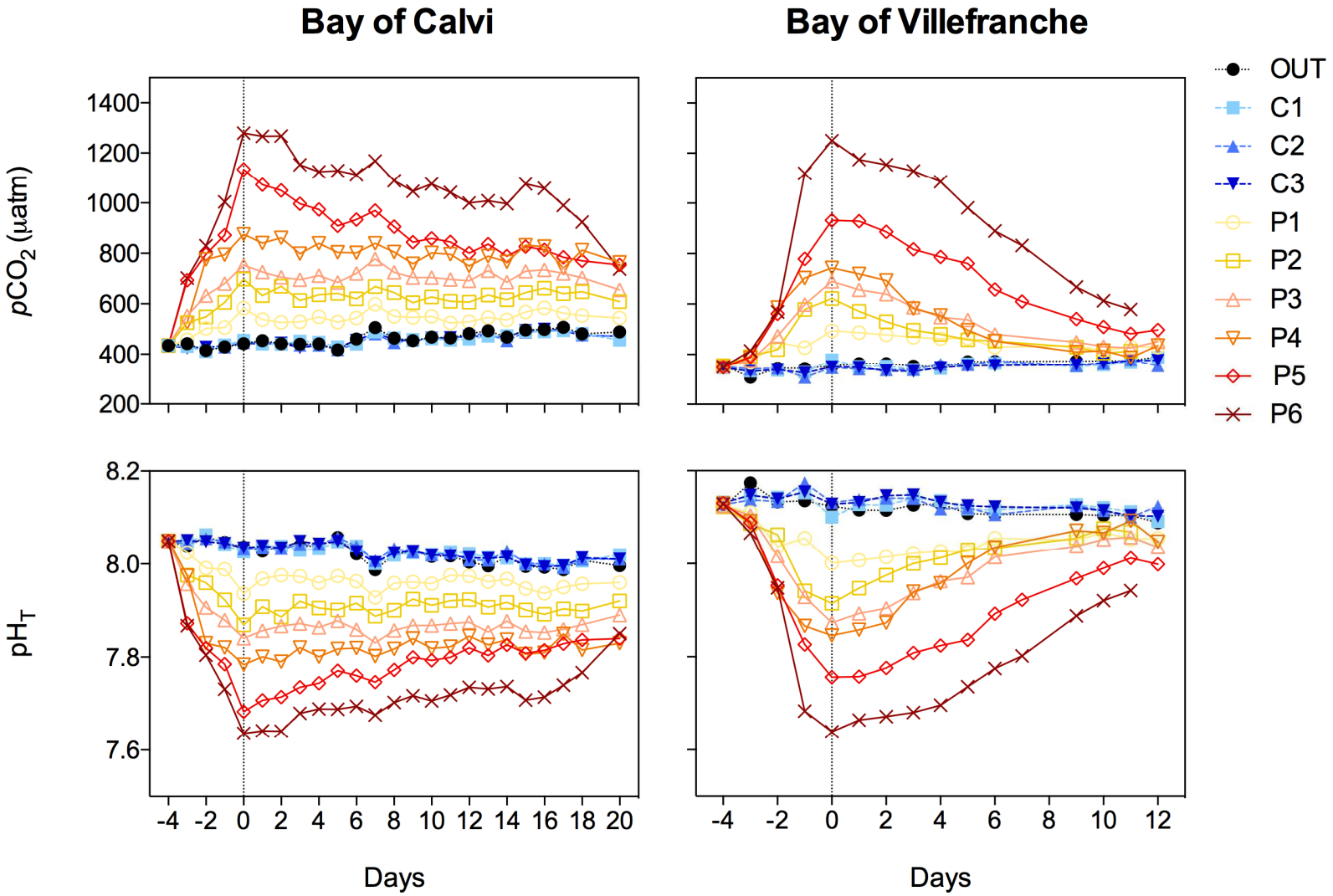
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total chl *a* | 27.2 | 0.45 | 156 | < 0.001 | S | + | < 0.001 |
| | | | | | $NH_4^+$ | + | 0.006 |
| Prasinophyceae | 13.8 | 0.24 | 157 | < 0.001 | $NO_3^-$ | + | < 0.001 |
| | | | | | S | + | < 0.001 |
| | | | | | I | + | < 0.001 |
| **Dinophyceae** | 19.5 | 0.36 | 156 | < 0.001 | $pCO_2$ | + | < 0.001 |
| | | | | | I | + | < 0.001 |
| | | | | | S | + | < 0.001 |
| | | | | | $NO_3^-$ | - | 0.001 |
| Cryptophyceae | 32.3 | 0.49 | 156 | < 0.001 | $NO_3^-$ | + | < 0.001 |
| | | | | | T | - | 0.001 |
| | | | | | I | + | 0.01 |
| **Haptophyceae** | 11.4 | 0.28 | 155 | < 0.001 | $pCO_2$ | + | < 0.001 |
| | | | | | I | + | < 0.001 |
| | | | | | $NO_3^-$ | + | 0.002 |
| Pelagophyceae | 19.4 | 0.48 | 153 | < 0.001 | S | + | < 0.001 |
| | | | | | I | + | < 0.001 |
| | | | | | T | + | 0.001 |
| | | | | | $PO_4^{3-}$ | + | 0.005 |
| Chlorophyceae | 104 | 0.66 | 158 | < 0.001 | S | + | < 0.001 |
| | | | | | T | + | 0.002 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total chl *a* | 22.5 | 0.51 | 100 | < 0.001 | I | - | < 0.001 |
| | | | | | S | - | < 0.001 |
| | | | | | $NO_3^-$ | - | < 0.001 |
| Prasinophyceae | 18.7 | 0.50 | 99 | < 0.001 | T | + | < 0.001 |
| | | | | | $NO_3^-$ | + | < 0.001 |
| | | | | | $NH_4^+$ | + | < 0.001 |
| Dinophyceae | 14.5 | 0.39 | 100 | < 0.001 | S | - | < 0.001 |
| | | | | | w | - | < 0.001 |
| Cryptophyceae | 58.5 | 0.77 | 99 | < 0.001 | S | - | < 0.001 |
| | | | | | $NO_3^-$ | - | < 0.001 |
| | | | | | T | - | < 0.001 |
| **Haptophyceae** | 20.3 | 0.48 | 100 | < 0.001 | T | - | < 0.001 |
| | | | | | $NO_3^-$ | - | < 0.001 |
| | | | | | $pCO_2$ | - | < 0.001 |
| Pelagophyceae | 10.6 | 0.27 | 101 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| Chlorophyceae | 12.2 | 0.39 | 99 | < 0.001 | $NO_3^-$ | + | 0.001 |
| | | | | | S | - | 0.004 |
| | | | | | T | + | 0.004 |
| | | | | | I | - | 0.005 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyanophyceae | 15.6 | 0.27 | 157 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| | | | | | $PO_4^{3-}$ | - | < 0.001 |
| Diatoms | 12 | 0.29 | 155 | < 0.001 | I | - | < 0.001 |
| | | | | | T | - | < 0.001 |
| | | | | | $PO_4^{3-}$ | - | < 0.001 |
| Flow cytometry | | | | | | | |
| *Prochlorococcus* | NA | | | | | | |
| *Synechococcus* | 18.7 | 0.44 | 85 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| | | | | | I | - | < 0.001 |
| Pico-eukaryotes | 12.8 | 0.40 | 84 | < 0.001 | $NO_3^-$ | - | < 0.001 |
| Nano-eukaryotes | NA | | | | | | |
| Particle flux | | | | | | | |

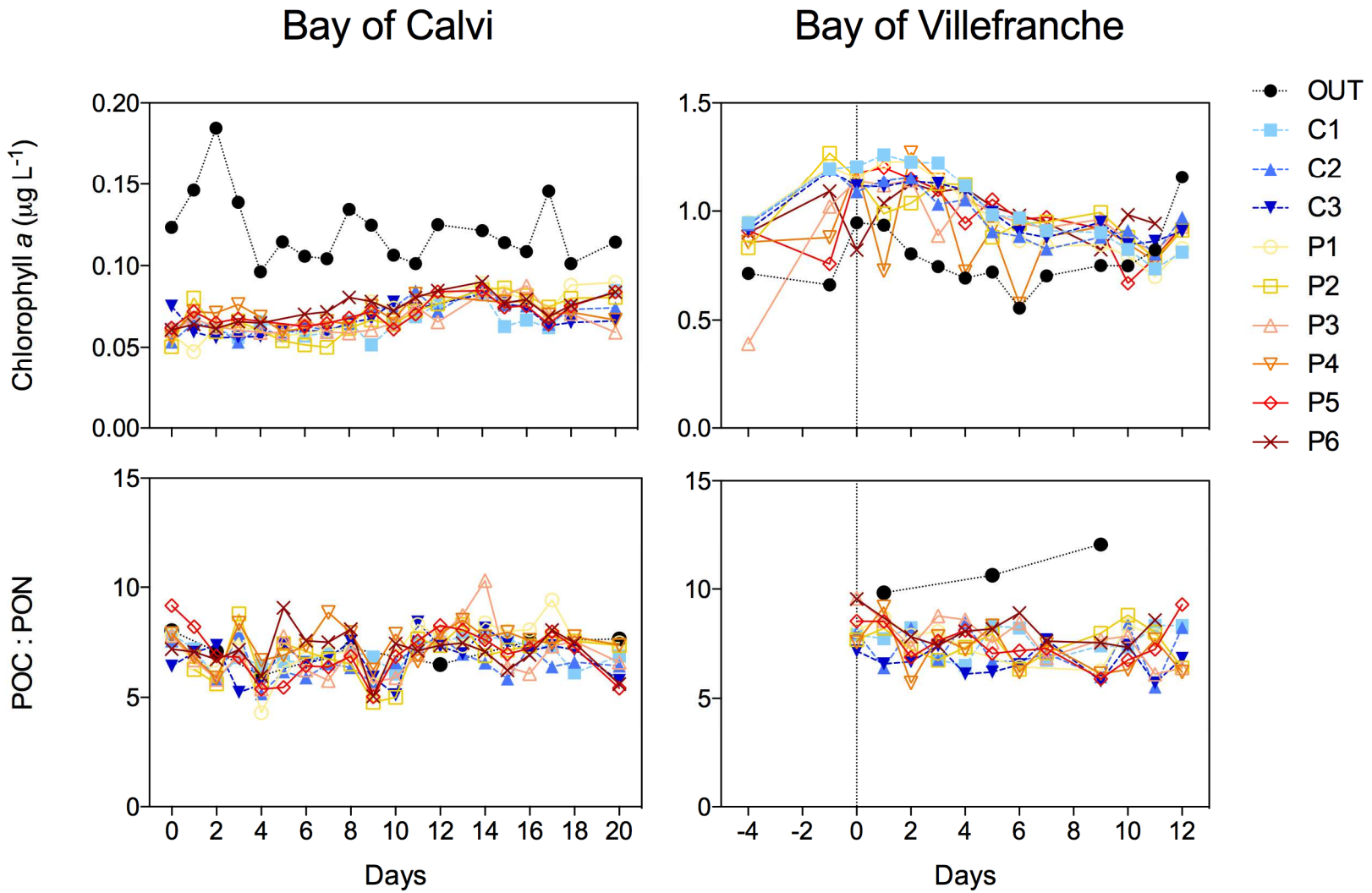
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyanophyceae | 132 | 0.83 | 101 | < 0.001 | S | + | < 0.001 |
| | | | | | T | + | < 0.001 |
| | | | | | $NO_3^-$ | + | < 0.001 |
| **Diatoms** | 40 | 0.45 | 100 | < 0.001 | S | - | < 0.001 |
| | | | | | $pCO_2$ | + | < 0.001 |
| | | | | | $NH_4^+$ | + | < 0.001 |
| | | | | | I | - | 0.006 |
| Flow cytometry | | | | | | | |
| *Prochlorococcus* | 17.8 | 0.49 | 66 | < 0.001 | S | + | < 0.001 |
| | | | | | T | + | < 0.001 |
| *Synechococcus* | 98.2 | 0.89 | 64 | < 0.001 | S | + | < 0.001 |
| | | | | | T | + | < 0.001 |
| | | | | | $NO_3^-$ | + | < 0.001 |
| | | | | | $NH_4^+$ | - | 0.003 |
| Pico-eukaryotes | 22.8 | 0.61 | 65 | < 0.001 | T | + | < 0.001 |
| | | | | | w | + | < 0.001 |
| | | | | | S | - | < 0.001 |
| | | | | | $NO_3^-$ | + | < 0.001 |
| | | | | | $NH_4^+$ | + | 0.005 |
| Nano-eukaryotes | 27.4 | 0.65 | 65 | < 0.001 | S | - | < 0.001 |
| | | | | | $NO_3^-$ | - | < 0.001 |
| | | | | | w | + | < 0.001 |
| | | | | | $NH_4^+$ | + | 0.006 |
| Particle flux | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | POC | 33.7 | 0.63 | 74 | < 0.001 | T | - | < 0.001 | POC | NS |
| | PON | 34.6 | 0.63 | 74 | < 0.001 | T | - | < 0.001 | PON | NA |
| | | | | | | $NO_3^-$ | + | 0.009 | | |
| | POC:PON | 22.9 | 0.46 | 75 | < 0.001 | S | + | < 0.001 | POC:PON | NA |



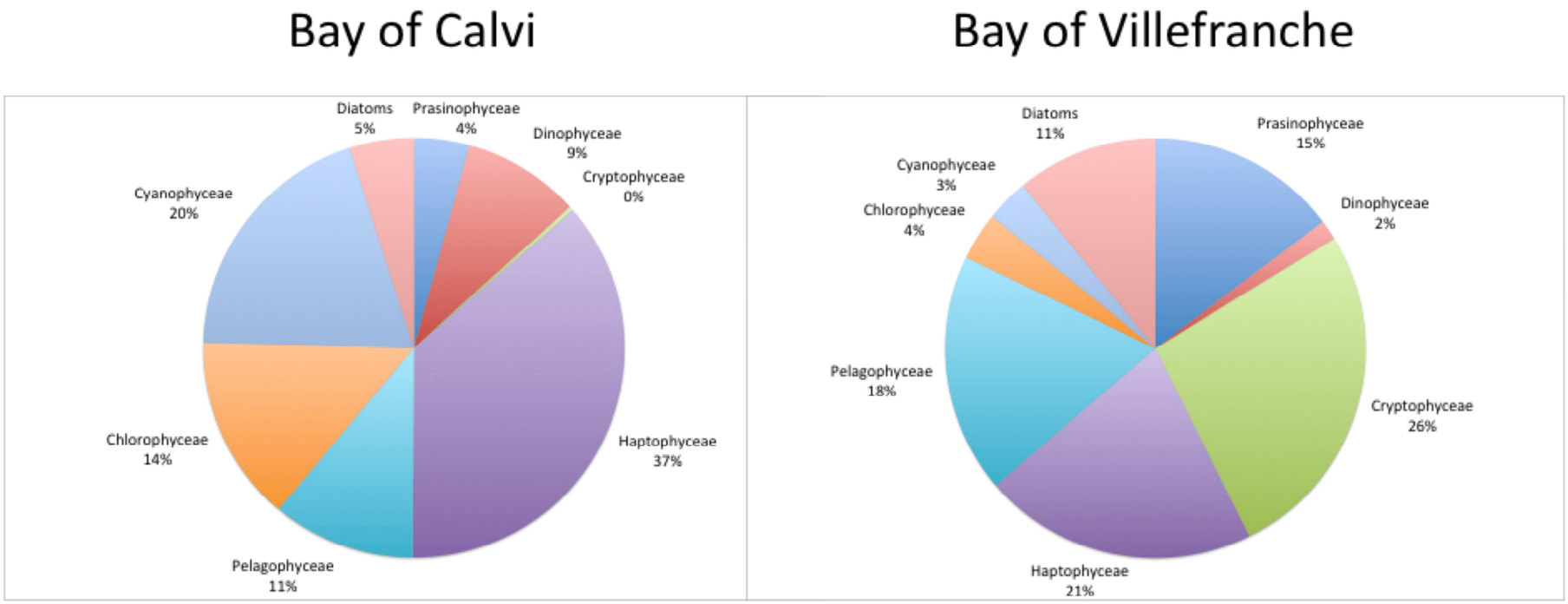

1019

1020 Fig. 1



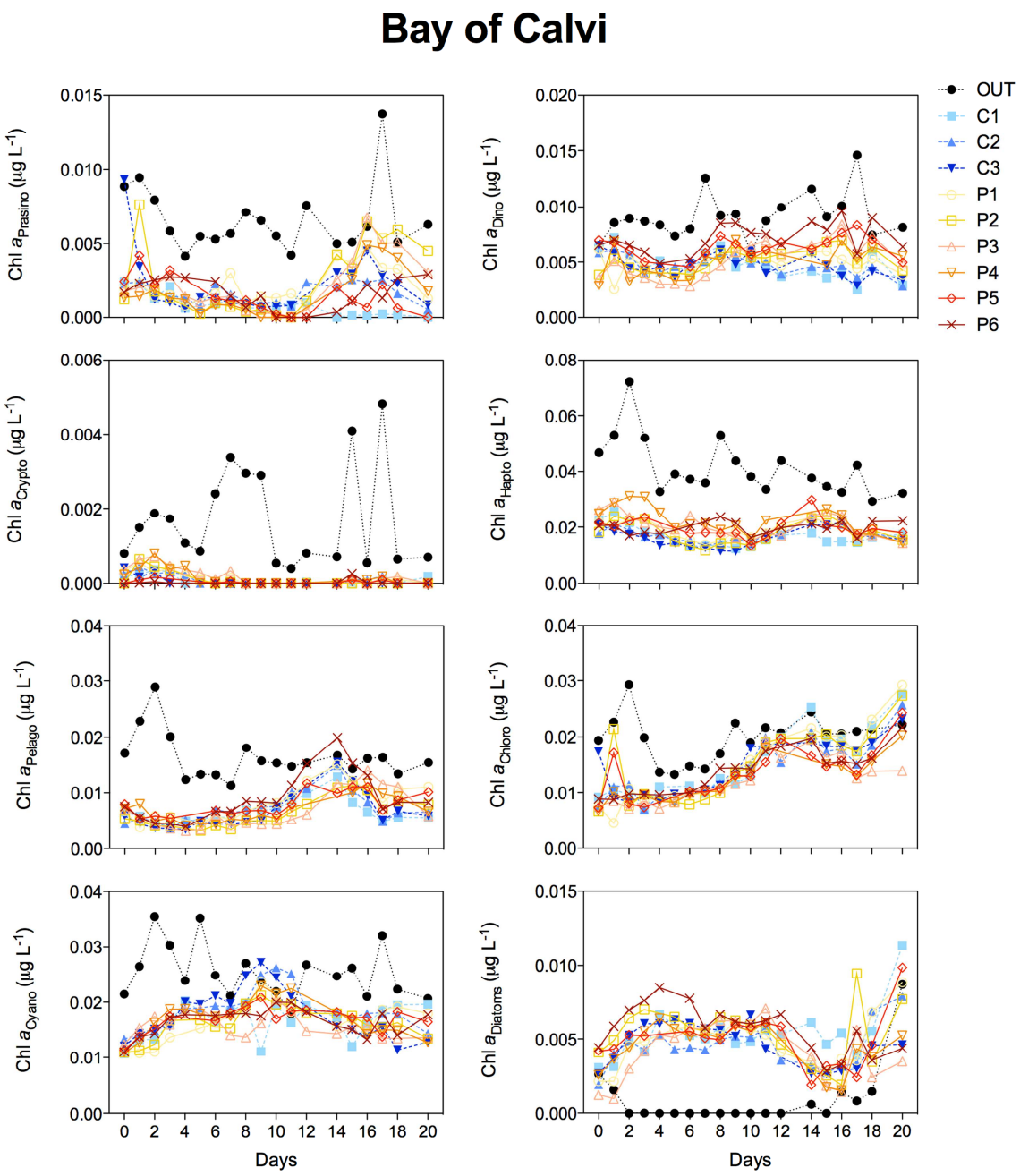

1021

1022 Fig. 2



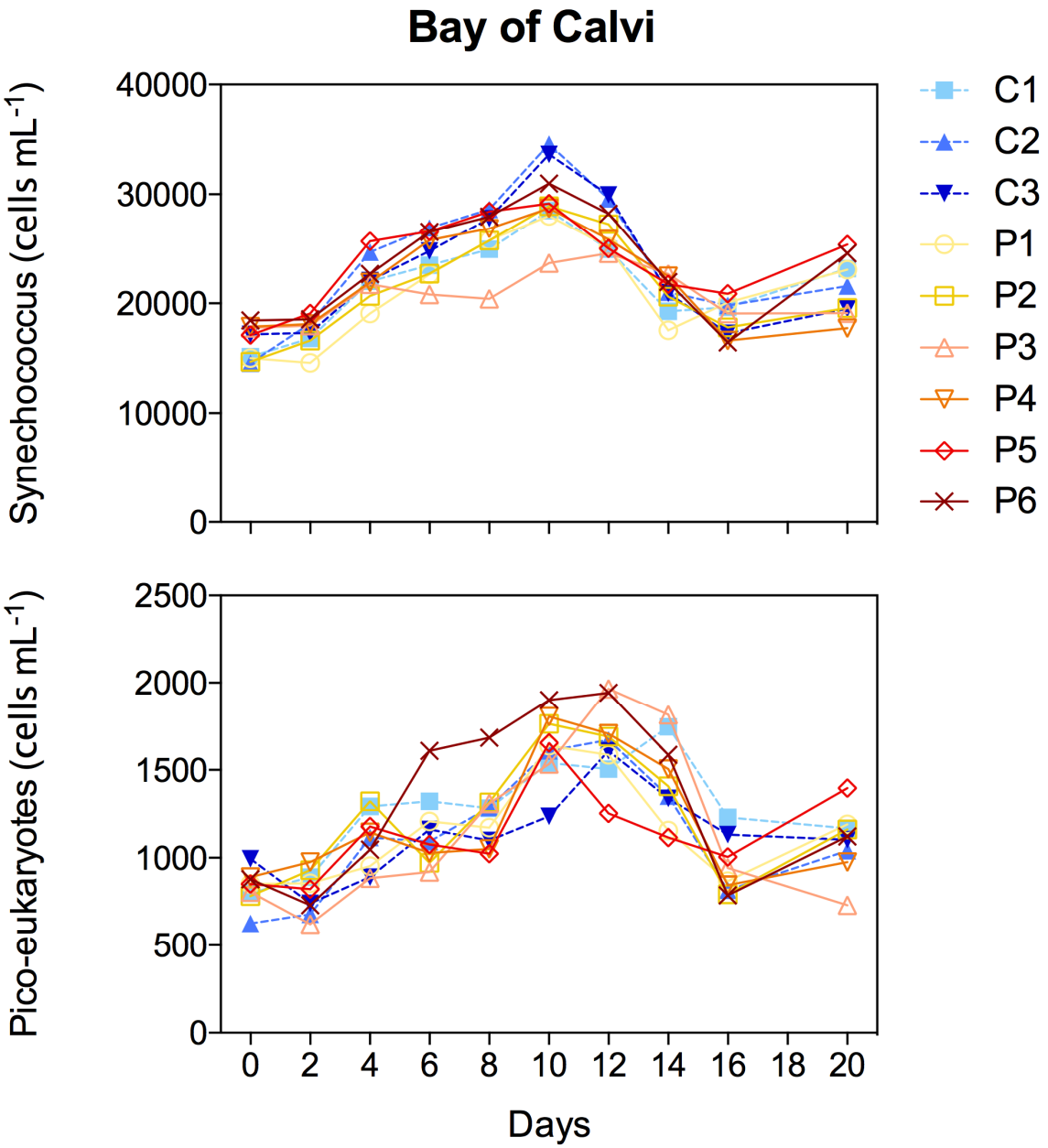

1023

1024 Fig. 3




1025

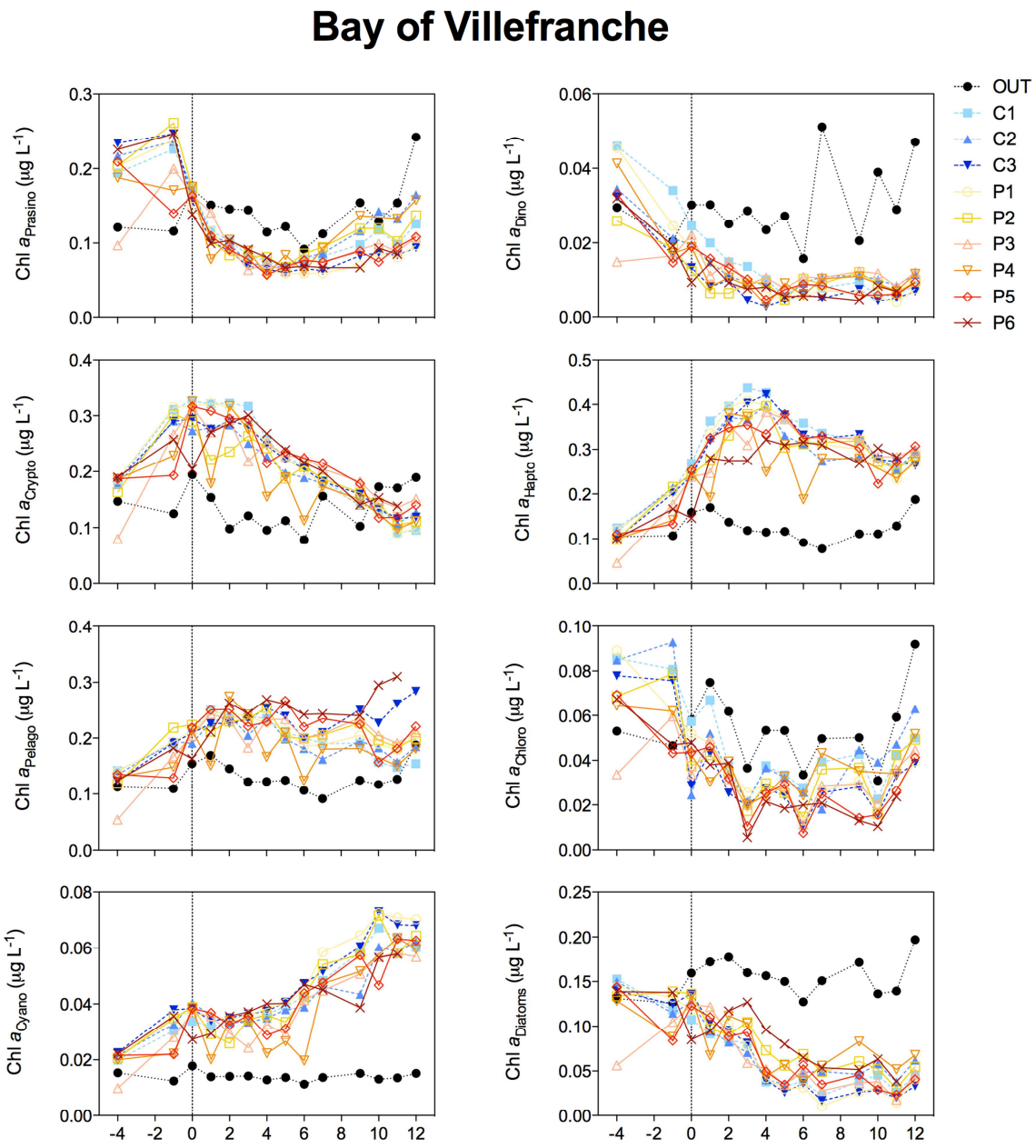
1026 Fig. 4




1027

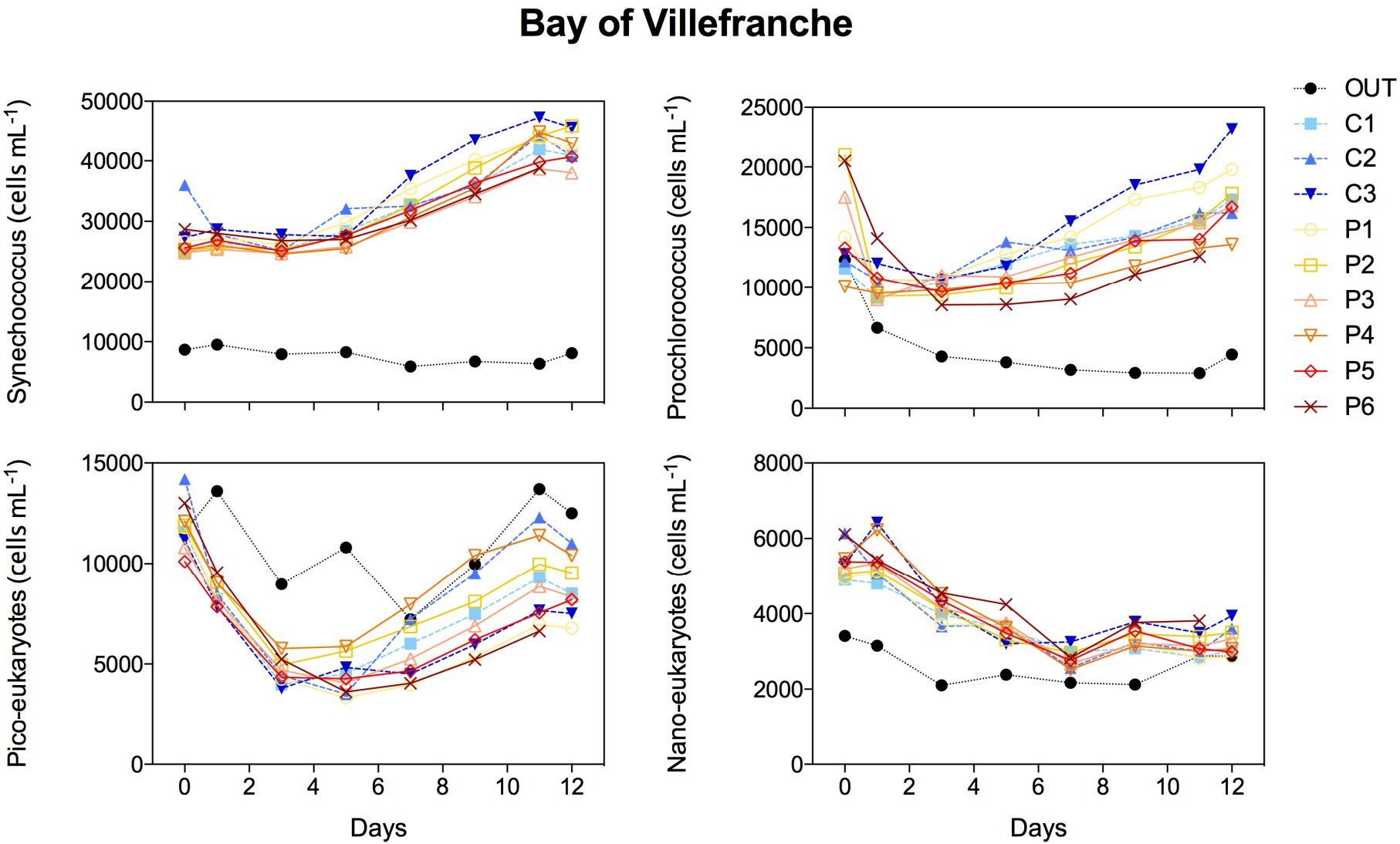
1028 Fig. 5

1029

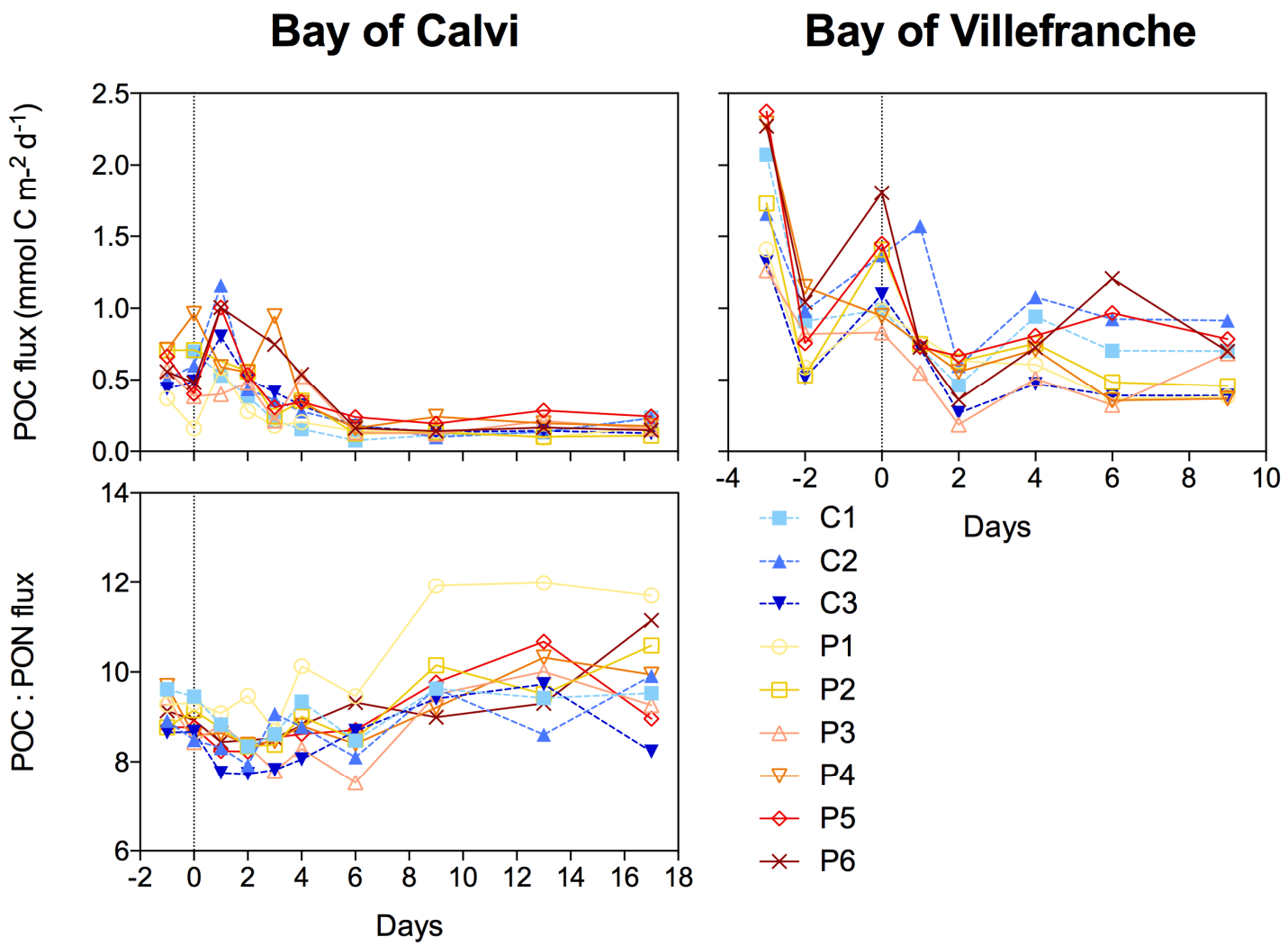



1030

1031 Fig. 6




1032
1033 Fig. 7




1034

1035 Fig. 8