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Response of plankton communities to ocean warming and acidification in the NW Mediterranean Sea

Laure Maugendre

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Université Pierre et Marie Curie

Ecole doctorale 129

Laboratoire d'Océanographie de Villefranche – Equipe B&B

Response of plankton communities

to ocean warming and acidification in the

NW Mediterranean Sea

Par Laure Maugendre

Thèse de doctorat en Océanographie Biologique

Dirigée par Frédéric Gazeau et Jean-Pierre Gattuso

Présentée et soutenue publiquement le 31 Octobre 2014

Devant un jury composé de :

Patrizia Ziveri, Professor, Universidad Autonoma de Barcelona (reviewer)

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Université Pierre et Marie Curie

Ecole doctorale 129

Laboratoire d'Océanographie de Villefranche – Equipe B&B

**Réponse de communautés planctoniques au
réchauffement et à l'acidification de l'océan en
Méditerranée du Nord-Ouest**

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Abstract

Plankton plays a key role in the global carbon cycle. It is therefore important to project the evolution of plankton community structure and function in a future high-CO₂ world. Several experimental results reported at the community level have shown increased rates of primary production as a function of increasing *p*CO₂ and few multi-driver experiments have been performed. However, the great majority of these experiments have been performed under high natural or nutrient-enriched conditions and very few data are available in areas with naturally low levels of nutrient and chlorophyll i.e. oligotrophic areas such as the Mediterranean Sea, although they represent a large and expanding part of the ocean surface.

Several approaches have been used during this thesis to investigate the effects of ocean warming and acidification on plankton communities in the NW Mediterranean Sea. One approach, restricted to the investigation of ocean acidification effects alone, was the use of mesocosms. In the Bay of Calvi (experiment #1; summer 2012 during 22 days), the community was very efficient in recycling nutrients and showed important regenerated production while in the Bay of Villefranche (experiment #2; winter/spring 2013 during 11 days) the community was characterized by a more autotrophic state and larger new production. A third experiment was set-up to investigate the combined effects of ocean acidification and warming in small containers in the Bay of Villefranche (experiment #3; March 2012; post-bloom conditions).

All experiments were conducted under low nutrient conditions with communities dominated by small species (e.g. haptophytes, cyanobacteria, chlorophytes). During the third experiment, biomass of populations decreased throughout the experiment (12 days), except cyanobacteria (mostly *Synechococcus* spp.) that significantly increased during that period. This increase was even more pronounced under elevated temperature, albeit the combination with elevated *p*CO₂ tended to limit this effect. For the three experiments, ocean acidification alone had no effect on any of the metabolic processes, irrespective of the methods used (O₂-LD, as well as ¹⁸O, ¹³C and ¹⁴C labelling) while during the multi-driver experiment #3, ocean warming led to enhanced gross primary production as measured by the ¹⁸O labelling technique. Specific biomarkers, polar lipid fatty acids, were used in combination with ¹³C labelling to assess group primary production rates. This confirmed that ocean acidification alone did not favour any phytoplankton group under our experimental conditions.

Based on our findings and on an extensive literature review, it appears that most (57 %) of the experiments performed to date have shown no effect of ocean acidification alone while ocean warming seem to have an effect on plankton composition and production. Furthermore, plankton biomass in ecosystems dominated by small phytoplankton species appears insensitive to elevated CO₂. It remains, for the moment, impossible based on these findings to provide a general concept on the effect of ocean acidification on plankton communities. However, it appears that ocean acidification will likely not lead to increased biomass and primary production rates for most communities, as it was previously anticipated. Furthermore, although warming will likely lead to increased primary production, it appears that small species with a low capacity for export will be favoured. If this proves to be a widespread response, plankton will not help mitigating atmospheric CO₂ increase through an enhancement of the biological pump.

Key words: ocean acidification, ocean warming, plankton community, primary production, oligotrophic area, Mediterranean Sea, stable isotope, mesocosm

Le plancton a un rôle crucial dans le cycle du carbone. Il est donc primordial de projeter son évolution dans le contexte de changement climatique. Une partie des résultats rapportés au niveau des communautés planctoniques montrent une stimulation de la production primaire avec l'augmentation de concentration en CO₂ et très peu d'expériences combinant plusieurs facteurs ont été faites. Qui plus est, les expériences ont été réalisées majoritairement dans des conditions naturellement élevées ou enrichies en sels nutritifs et très peu de données existent dans les zones naturellement pauvres en nutriments et chlorophylle *a*, c'est à dire dans les zones oligotrophes telles que la mer Méditerranée, bien que ces régions représentent une surface importante et en expansion de la surface de l'océan.

Plusieurs approches ont été utilisées au cours de cette thèse pour étudier les effets du réchauffement et de l'acidification de l'océan sur des communautés planctoniques dans le NO de la Méditerranée. Une des approches, restreinte à l'effet de l'acidification seule, a été l'utilisation de mesocosmes. En Baie de Calvi (expérience #1; été 2012 sur 22 jours) la communauté étudiée présentait un efficace processus de recyclage des sels nutritifs ainsi qu'une production régénérée importante alors que dans le Baie de Villefranche (expérience #2; hiver/printemps 2013 durant 11 jours) la communauté était caractérisée plutôt par un système autotrophe et par une production nouvelle dominante. Une troisième expérience a été réalisée pour étudier les effets synergétiques de l'acidification et du réchauffement de l'océan (expérience #3; March 2012; post-bloom).

Toutes les expériences ont ainsi été menées dans des conditions de faibles concentrations en sels nutritifs avec des communautés dominées par des petites espèces phytoplanctoniques telles que des haptophytes, cynobactéries et chlorophytes. Lors de l'expérience #3, toutes les populations ont décliné au cours de l'expérience (12 jours) à l'exception des cyanobactéries (principalement *Synechococcus* spp.) qui ont significativement augmenté durant cette période. Cette augmentation était d'autant plus prononcée dans les conditions de température plus élevée, bien que l'augmentation concomitante de CO₂ ai eu tendance à limiter cet effet. Pour les trois expériences, l'acidification de l'océan seule n'a pas montré d'effet sur les taux métaboliques quelque soit la méthode utilisée (O₂-LD, marquage au ¹⁸O, ¹³C et ¹⁴C) alors que durant l'expérience #3, les conditions élevées en température ont favorisé la production brute déterminée par la méthode de marquage ¹⁸O. Des biomarqueurs spécifiques, les acides gras des lipides polaires, utilisés de façon combinée avec du marquage au ¹³C a permis la détermination des productions primaires par groupe. Ceci a confirmé que l'acidification de l'océan seule n'a pas particulièrement favorisé un groupe phytoplanctonique par rapport à un autre dans nos conditions expérimentales.

Basé sur nos résultats et sur une revue de littérature, il apparaît que la plupart des expériences (57 % des études) réalisées jusqu'à maintenant n'ont pas montré d'influence notoire de l'acidification de l'océan seule sur les communautés planctoniques, alors que le réchauffement de l'océan semble avoir plus d'effet sur la composition et la production planctonique. De plus, la biomasse dans les écosystèmes dominés par des petites espèces de phytoplancton semble être insensible à l'augmentation de CO₂. A l'heure actuelle, il est impossible, basé sur ces résultats, de fournir un concept général de l'effet de l'acidification de l'océan sur les communautés planctoniques. Cependant il semble que l'acidification n'augmentera pas la biomasse et la production primaire pour la majorité des communautés. Qui plus est, bien que le réchauffement de l'océan pourrait augmenter la production primaire, il semble que les petites espèces présentant de faibles capacités d'export pourraient être favorisées. Si cela s'avère être une réponse générale, le plancton pourrait ne pas participer à l'atténuation de l'augmentation de CO₂ atmosphérique par une plus forte pompe biologique.

Mot-clés: acidification de l'océan, réchauffement de l'océan, communauté planctonique, production primaire, mer Méditerranée, isotope stable, mesocosme

A Pierre Rabhi,
To Pierre Rabhi,

“Dare is to lose foot momentarily,
not to dare is to lose oneself.”

« Oser c'est perdre pied momentanément,
ne pas oser c'est se perdre soi-même. »

Kierkegaard

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Glossary

BATS: Bermuda Atlantic Time-series Station

CCM: Carbon Concentration Mechanism

CMIP5: Coupled Model Intercomparison Project (2010-2014)

CR : Community Respiration

CTD : Conductivity Temperature Depth

DYFAMED: DYnamique des Flux Atmospherique en MEDiterrannée (Dynamic of atmospheric fluxes in Mediterranean Sea)

EPOCA : European Program Ocean Acidification

GPP : Gross Primary Production

HNLC : High Nutrient, Low Chlorophyll

HOT : Hawaii Ocean Time-series

HPLC : High Performance Liquid Chromatography

IPCC: Intergovernmental Panel on Climate Change

LNLC : Low Nutrient, Low Chlorophyll

MedSea : Mediterranean Sea Acidification European project

MS: Mediterranean Sea

NCP : Net Community Production

OA : Ocean Acidification

OW : Ocean Warming

$p\text{CO}_2$: carbon dioxide partial pressure

PeECE: Pelagic Ecosystem CO_2 Enrichment

PP : Primary Production

UKOA: United Kingdom Ocean Acidification research programme

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Chapter I

Introduction to the plankton community in the Anthropocene

1. The Anthropocene

A working group of researchers of the International Union of Geological Science (IUGS) is preparing to release a report in 2016 to state whether the Earth has entered a new geological era called the Anthropocene (from *anthrôpos* 'human' and *kainos* 'recent') and, if so, when this new era was reached (Crutzen and Stoermer, 2000). Simultaneously, the Intergovernmental Panel on Climate Change (IPCC) has produced a fifth report on climate change. The conclusions of this report are clear: human activities are responsible for environmental perturbations caused by carbon dioxide (CO₂) and other greenhouse gases. Furthermore, it states that emissions at an unprecedented rate are transgressing planetary boundaries for the safety of humanity (Rockstöm et al., 2009).

The definition of the Anthropocene or anthropogenic global change (i.e. changes related to human activities) cover several aspects such as land use changes, deforestation or changes in biogeochemical cycles such as nitrogen cycle but also expansion of mankind in number and exploitation of Earth's resources (Crutzen and Stoermer, 2000). However, all those aspects of the Anthropocene cannot be presented in details in this Introduction. Here two aspects of the effect of human activities are emphasized and are specifically related to excessive CO₂ emissions by human activities in the atmosphere and affecting the ocean.

Indeed, since the industrial revolution, human activities release significant amounts of CO₂ to the atmosphere through burning of fossil fuel and land use changes (Figure I-1a) leading to atmospheric and ocean warming (Figure I-1b; Levitus et al., 2001). Depending on human's development and behaviour, different CO₂ emission scenarios can be made and an increase of 0.6 to 5 °C in the ocean for 2100 have been established through several models, although projections are difficult due to regional and temporal (from daily to inter-annual) variability.

Besides ocean warming by heat transfer, the ocean absorbs about 25% of the CO₂ emissions (Le Quéré et al., 2013). Carbon dioxide, as other gases, exchanges with its dissolved form at the ocean surface forming carbonic acid (H₂CO₃), a weak acid that rapidly dissociates to bicarbonate ions (HCO₃⁻), carbonate ions (CO₃²⁻) and protons (H⁺; Figure I-2). The sum of seawater CO₂, HCO₃⁻ and CO₃²⁻, is termed dissolved inorganic carbon (C_T).

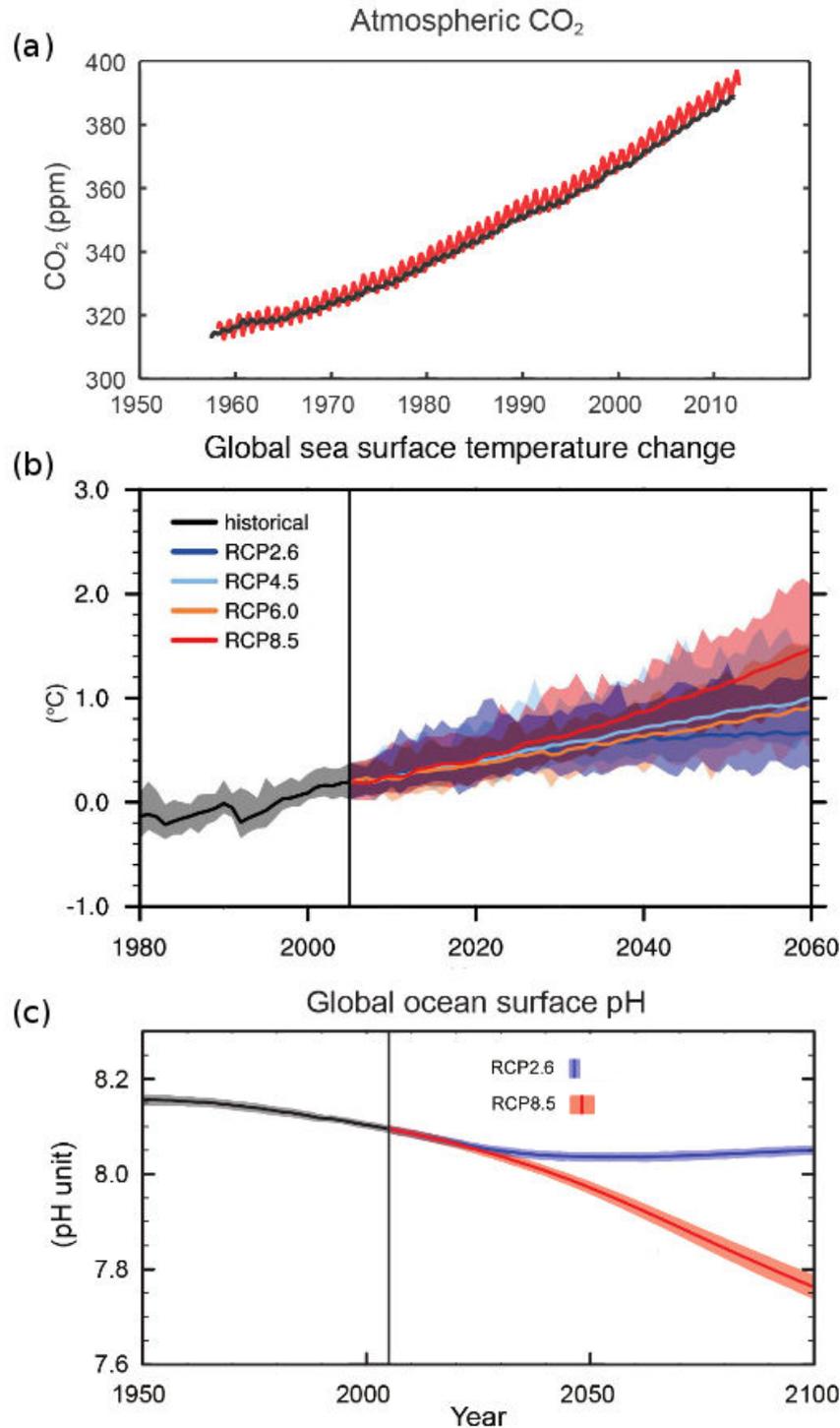


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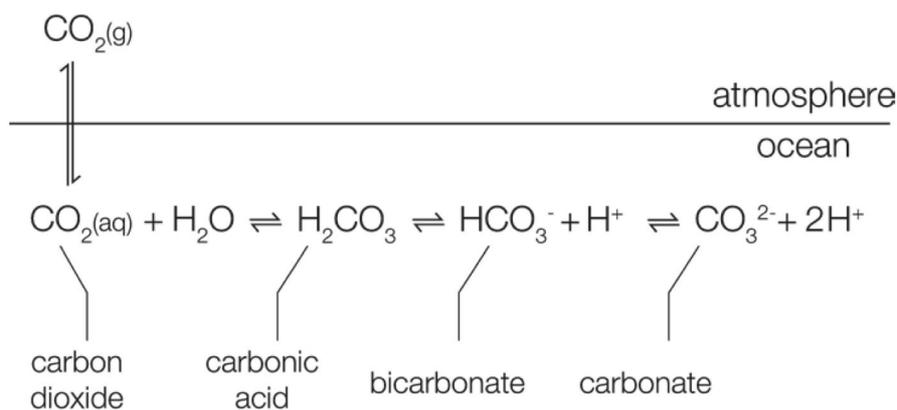


Figure I-2. Atmosphere-Ocean CO₂ exchanges and carbonate chemistry equilibrium. Source: Rokitta (2012).

These chemical species are at equilibrium under specific conditions of temperature, salinity and pressure. A change in pH or CO₂ alters the speciation and preponderance of the different elements. At present seawater pH (~8.1 pH units on the total scale), the major part of C_T is present in bicarbonate form (91%; Figure I-3). Due to the increase in atmospheric CO₂ partial pressure (*p*CO₂), more CO₂ is dissolved into the ocean, increasing the concentration of C_T, CO₂, HCO₃⁻ and H⁺ and decreasing the concentrations of CO₃²⁻ and pH. This process is known as ‘ocean acidification’ (OA; Doney et al., 2009, Gattuso and Hansson, 2011). It is estimated that pH of surface waters has decreased by 0.1 pH units since 1900 and that it will decrease by an additional 0.06 to 0.34 pH units by 2100 depending on the CO₂ emission scenario considered (IPCC, 2013 in Chapter 3; Figure I-1c). CO₂ dissolution in the ocean is sensitive to temperature, dissolution being larger in cold than in warm waters, thus future ocean warming will decrease the capacity of the oceans to store CO₂.

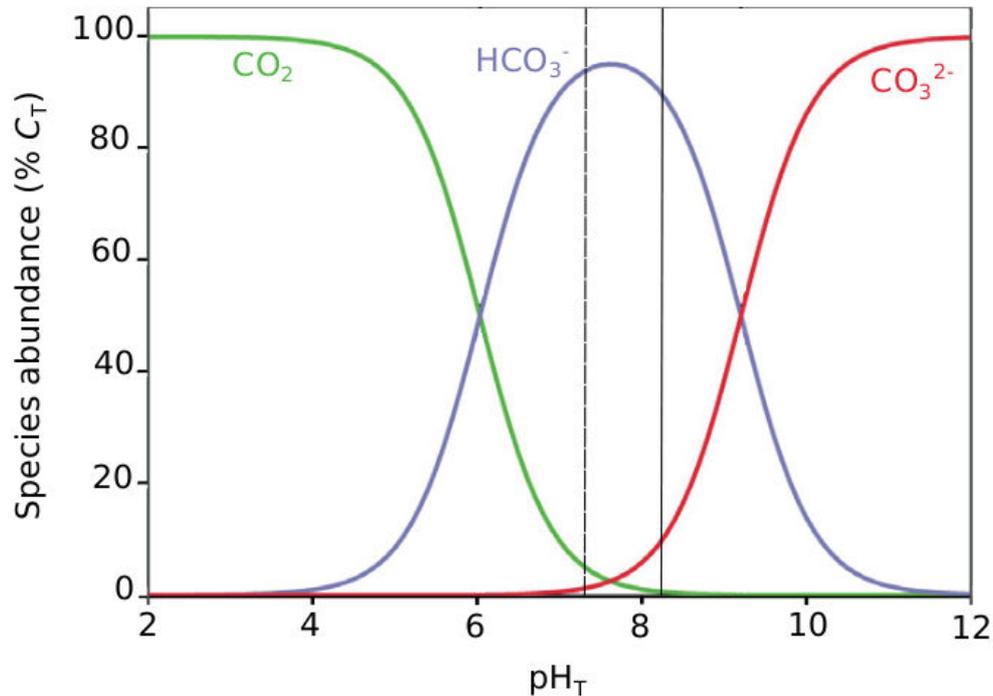


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The rapid flow of publications on OA prompted the compilation of an expert survey by Gattuso et al. (2012) which assessed the current knowledge and confidence level for several statements regarding ocean acidification and its effect on the ocean. While the chemical process of ocean acidification is very well documented leading to very high levels of confidence, the biological and biogeochemical responses are associated with much lower confidence levels. It appears that there is a **lack of knowledge regarding the response of the plankton community to ocean acidification in various regions of the ocean, especially in the context of a concomitant warming (multi-stressors studies).**

2. Carbon pump

The ocean is the largest active reservoir of carbon on Earth and absorbs about 2.6 ± 0.5 Pg C yr⁻¹ (Le Quéré et al., 2013). Carbon uptake is controlled by two mechanisms: the solubility and biological pumps (Figure I-4). The solubility pump is the process mentioned above: CO₂ dissolves in the ocean and is sequestered to the ocean interior by water masses sinking at high latitudes. The biological pump is the transport of organic matter from the surface to the deep sea. It is considered that about 50% of the global Earth primary production occurs in the ocean (Field et al., 1998), despite the fact that it represents less than 1% of the global photosynthetic biomass (Antoine et al., 1996; Behrenfeld and Falkowski, 1997). **Oceanic primary production has, therefore, a key role in carbon cycle and climate regulation.**

The majority of carbon fixation in the surface layer is performed by pelagic phytoplankton, which uses CO₂ and converts it to organic matter (OM) through photosynthesis (~ 50 PgC yr⁻¹; Field et al., 1998). This reaction is powered by light and requires nutrients (nitrogen and phosphate being the main macro-nutrients), following the simplified photosynthetic reaction:



In addition, the production of calcareous structures by many planktonic or benthic species in the ocean counteracts the CO₂ sequestration during photosynthesis and calcium carbonate production represent about 0.8-1.4 PgCaCO₃ yr⁻¹ (Feely et al., 2004). Indeed, calcifying organisms use bicarbonate ions to build their skeleton and the production of calcium carbonate releases CO₂:



The organic matter produced in the surface layers can be exported to the deep sea. However, heterotrophs (e.g. bacteria, flagellates) remineralise this OM in surface layers through respiration, consuming O₂ and releasing CO₂ back to seawater. Although most of the community respiration is due to bacteria, it must be stressed that part of the respiration is realised by autotrophs during both light and dark periods. It has been estimated that about 70% of the OM produced in the mixed layer is recycled while 30% is exported to the deep sea (Falkowski et al., 1998) where it is partially remineralised by bacteria. Finally, only 1-3% of the OM produced in the surface layer is definitely buried in the sediments (De La Rocha and Passow, 2007) while approximately 13-30% of the CaCO₃ produced is ultimately stored in the sediment (Feely et al., 2004; Sarmiento and Gruber, 2006).

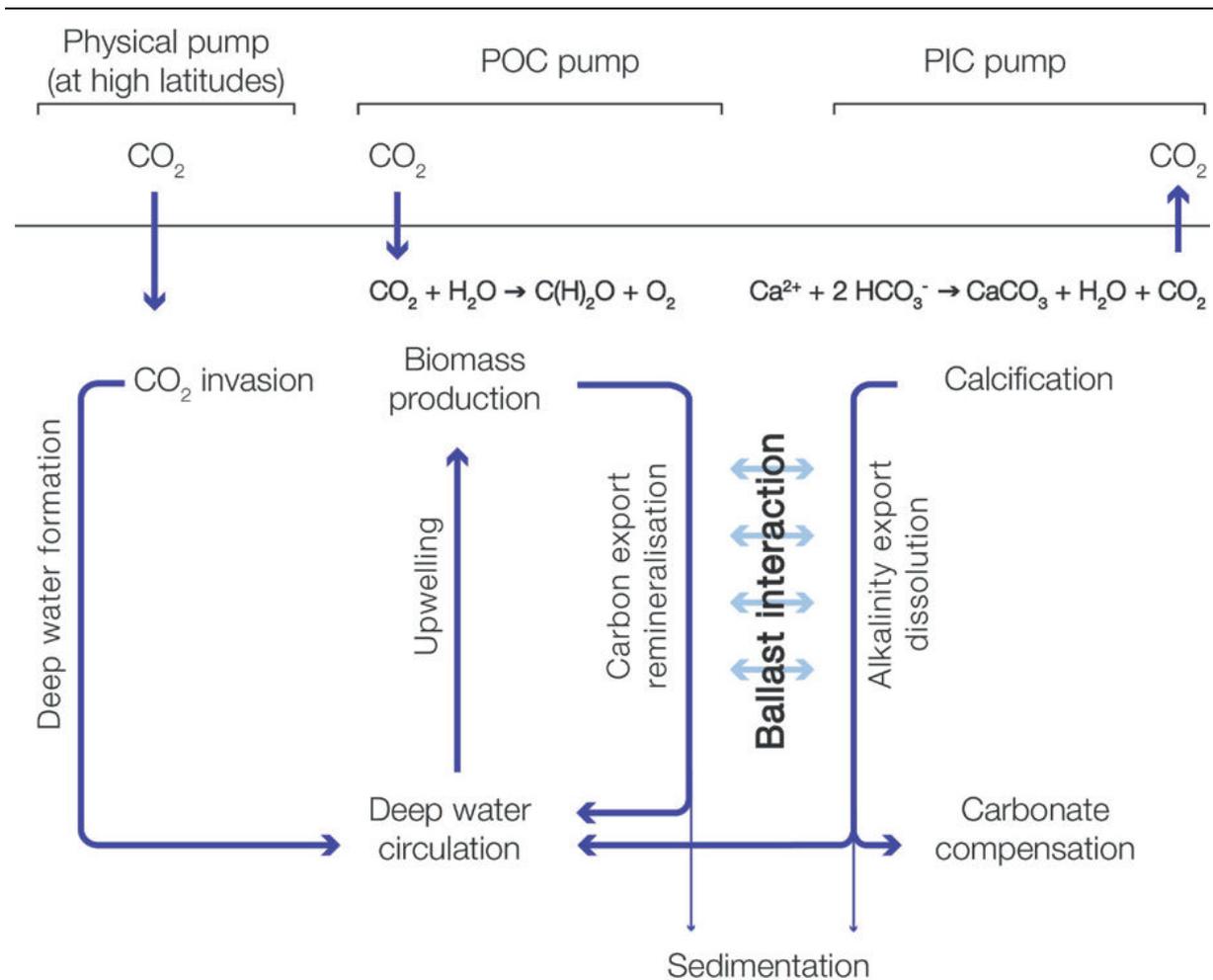


Figure I-4. Representation of the solubility pump, the biological pump and carbonate pump. From Rokitta (2012).

The surface ocean is not homogeneous in terms CO_2 exchanges with the atmosphere. The solubility capacity depends on the ocean surface temperature as CO_2 dissolves more in cold than warm waters. In addition, the potential for carbon sequestration also depends on the metabolic status of the plankton community in the surface mixed layer, controlled by the balance between community gross primary production (GPP) and respiration (CR), i.e. the net community production (NCP) defined as the production of organic matter after it has been respired by all plankton communities ($\text{NCP} = \text{GPP} - \text{CR}$, if CR is expressed as a positive process). An ecosystem is autotrophic, and potentially a CO_2 sink for the atmosphere, when GPP exceeds CR ($\text{NCP} > 0$). Conversely, in a heterotrophic system, CR exceeds GPP ($\text{NCP} < 0$) with potentially a source of CO_2 for the atmosphere. **Primary production and respiration can push ecosystems towards being CO_2 sinks or sources. However, the ecosystem metabolic state does not always imply a air-sea CO_2 flux as it depends on the CO_2 partial pressure at the air-sea interface** (Gattuso et al., 1998).

The capacity for producing organic matter in the surface layer depends on environmental conditions such as temperature, water-column structure (mixed *vs.* stratified), irradiance and nutrient availability. In the classical plankton food-web, described as the production of phytoplankton species grazed by zooplankton which are subsequently consumed by higher trophic levels, high nutrient concentrations are required. In addition to this food web, the microbial loop describes the use of dissolved organic carbon (DOC) released by phytoplankton (about 10-15 % of the particulate primary production; Baines and Pace, 1991) and zooplankton, as a substrate for bacterial growth and leading to the recycling of nutrients. Classic food-web and microbial loop exist as a continuum of trophic structure and the predominance of one path relative to the other depends on the nutrient availability (Legendre and Rassoulzadegan, 1995) that also influence the metabolic balance of the ecosystem. The remineralisation by bacteria is also subject to temperature control by an inverse function (Rivkin and Legendre, 2001) and therefore influences scope for carbon export to the deep-sea. Finally, the capacity for carbon sink also depends on the phytoplankton community composition as phytoplankton species with calcium carbonate (e.g. coccolithophores) or silicate (e.g. diatoms) structures have better ballast properties and are fast-sinking particles (Klaas and Archer, 2002).

3. The evolution of plankton community in the Anthropocene

The ongoing environmental perturbations such as ocean acidification and warming could have profound effects on the functioning of plankton community. As a result, the strength of the biological pump could be affected, thereby altering the carbon storage capacity of the ocean

3.1 Effect of ocean warming

Temperature exerts a positive effect on phytoplankton metabolic rates as observed in laboratory culture (Eppley, 1972) and at sea (Regaudie-de-Gioux and Duarte, 2012), although a recent study suggests that phytoplankton carbon-specific production rates mostly depend on nutrient supply rather than on temperature (Marañón et al., 2014). However, photosynthesis and respiration exhibit different sensitivities to temperature. Phytoplankton growth and photosynthesis are less affected by a temperature increase (irradiance and nutrient availability exert tighter controls) than bacterial and heterotrophic respiration (López-Urrutia et al., 2006).

Thus, warmer conditions should increase respiration and therefore altering carbon cycling by promoting heterotrophy.

However, experimental evidence for this process is still missing and contradictory results have been reported. For instance, in Kiel Fjord (Germany) the effect of temperature has been investigated during two mesocosm experiments. During the first experiment, enhanced respiration was measured in warmer treatments, diminishing the C_T drawdown. Additionally, a shift toward a larger accumulation of dissolved organic carbon (DOC) (Wohlers et al., 2009) and higher C:N ratio of the dissolved organic matter (DOM) in warmer treatments (Engel et al., 2011) were found. In contrast, in the second experiment, C_T uptake as well as particulate organic carbon (POC) and DOC increased in the warmer treatments (Taucher et al., 2012). The differences in C_T drawdown during these two experiments were attributed to the different species of diatoms present in the community (*Skeletonema costatum* vs. *Dactyliosolen fragilissimus*), but could also be due to differing irradiance and temperature levels.

Plankton species have different metabolic thermal optima and a rapid change of average temperature could cause shifts in the community structure with some species benefiting from warmer conditions and adapting better than others (Lürling et al., 2013). Mesocosms and *in situ* data show that small species are favoured under warmer conditions (Sommer and Lengfellner, 2008; Morán et al., 2010; Peter and Sommer, 2012; Daufresne et al., 2009). This would have consequences on carbon export efficiency, as some phytoplankton species (e.g. diatoms and coccolithophores) have better ballasting properties than others. In addition, not all phytoplankton have the same food quality (lipids content and stoichiometric ratios) and therefore energy transfer capacity to higher trophic levels (zooplankton, fishes) differs (Dickman et al., 2008) with, for example, diatoms (large species) presenting better food quality than cyanobacteria (Müller-Navarra et al., 2000).

A full understanding of the effect of ocean warming on the plankton community, requires to consider both the direct effect of increased temperature on metabolic rates, and the indirect effect due to nutrient depletion in surface layers, as well as increased irradiance at high latitudes, caused by stronger stratification (Behrenfeld et al., 2006; Lewandowska et al., 2014). Indeed, satellite observations reported a decline of ~ 1% of the global median per year in surface plankton biomass during the last decade (Boyce et al., 2010). However, faster nutrient remineralisation by bacteria could offset the decrease in phytoplankton biomass by earlier bacterial activity peak after phytoplankton bloom, that tighten the coupling between phytoplankton and bacteria (Hoppe et al., 2008). **Temperature is recognized as a major**

parameter controlling plankton community structure and dynamics and there are still uncertainties on how the plankton community will evolve in the future warmer ocean. Finally, it is very likely that, as the efficiency of the carbon pump and its evolution in a warmer ocean seems to be closely related to nutrient regime and community composition (Boyce et al., 2010; Taucher and Oschlies, 2011), important regional variations will be observed in the coming decades.

3.2 Effect of ocean acidification

Higher levels of CO₂ in seawater lead to an ocean acidification, an environmental perturbation that could also affect phytoplankton metabolism. Phytoplankton acquires dissolved inorganic carbon to produce organic matter, using CO₂ as a substrate for photosynthesis despite the low affinity for CO₂ of rubilose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), a key enzyme involved in photosynthetic carbon fixation (Raven and Johnston, 1991) and limited diffusion capacities of CO₂ in water. To overcome these limitations, marine phytoplankton have developed carbon concentrating mechanisms (CCMs; Giordano et al., 2005) to raise CO₂ concentrations in the vicinity of RuBisCO involving carbonic anhydrase activities or bicarbonate transport through the cell for example (Reinfelder, 2011). An increase in CO₂ would increase the diffusion of CO₂ in the cells and may therefore benefit primary producers by lowering the energy cost of carbon acquisition, increasing CO₂ diffusion through the cell membranes (Raven et al., 2005) and reducing CO₂ leakage (Rost et al., 2006). Different CCMs have been developed by phytoplankton species varying within and among groups, it is therefore expected that organisms will be differently affected by CO₂ increase, potentially causing shifts in the plankton community composition (e.g. Rost et al., 2008).

3.2.1. Single cells cultures

The majority of early laboratory experiments performed on single species have shown enhanced carbon fixation (e.g. Buitenhuis et al., 1999; see Riebesell and Tortell, 2011 for comprehensive review on this section) however some species, such as coccolithophore strains, have shown a neutral (e.g. Langer et al., 2006) or inhibitive effect under nitrate limitation (e.g. Sciandra et al., 2003). Coccolithophores have been more studied than other species for the impact of ocean acidification on calcification, with decreases in calcification rates (e.g. Riebesell et al., 2000) observed in most of the studies, although contradictory results showing neutral or enhanced calcification have also been reported (e.g. Iglesias-

Rodriguez et al., 2008). Differences in the strains used have been shown to be critical and repetitions of experiments on some strains have not always led to the same results (see Riebesell and Tortell, 2011 for details).

3.2.2. Community studies

The contradictory results obtained at the species level indicated that extrapolation from monocultures to assemblages is not straightforward. The investigation of the effect of ocean acidification at community level is therefore necessary.

Initial experimental work at the community level has reported an increase (~ 15 %) of ^{14}C fixation under high $p\text{CO}_2$ conditions (Hein and Sand-Jensen, 1997) in the South Atlantic Ocean. The first large mesocosm experiments (> 10 m³) performed in the North Sea (PeECE 2001, 2003 and 2005) have shown different responses. Only one of these three experiments have shown an increase in primary production under high $p\text{CO}_2$ conditions (Egge et al., 2009) while no change in primary production was found in the first and second experiments (Delille et al., 2005 for PeECE I; unpublished data for PeECE II, see Egge et al., 2009). Other experiments carried out in different oceanic regions, with different incubation volume, have led to increased primary production (e.g. Tortell et al., 2008) or to no effect (e.g. Tortell et al., 2002; Yoshimura et al., 2013). The recent Svalbard mesocosm experiment showed no clear trend in net community production and community respiration over the whole period (Silyakova et al., 2013; Tanaka et al., 2013), but found decreased NCP when considering only the post-bloom period. However, ^{14}C carbon fixation increased with increasing $p\text{CO}_2$ levels (Engel et al., 2013). In this experiment, the community response was not straightforward and possibly related to the change in community composition over the different phases (before and after nutrient addition). However, the different methods for measuring primary production did not show the same results, so the conclusions of this experiment should be regarded with caution, although they reveal the complexity of the community metabolic response to ocean acidification under different physiological states conditioned by nutrient availability.

It is thought that species with less efficient CCMs under present day CO_2 concentrations should benefit more from increased CO_2 , while species with efficient CCMs should be less affected (Rost et al., 2008; Low-Décarie et al., 2014). Studies focusing on the effect of increased $p\text{CO}_2$ on community composition have also shown some contradictory results, with either a shift towards more diatoms (Tortell et al., 2002; 2008), towards less silicified species and smaller species, or no change (e.g. Nielsen et al., 2012; Yoshimura et al.,

2013). In addition, Yoshimura et al. (2013) investigated the ocean acidification response at two sites in the North Pacific. In the Bering Sea, a decrease in dissolved organic carbon production at high $p\text{CO}_2$ was found, while at the second site in the Pacific no CO_2 effect was found, probably due to different community compositions.

The results and findings of the experiments performed in close-to-*in-situ* conditions (mesocosms or on-deck bottle incubations) have generated contradictory and ambiguous responses, failing to establish consistent responses to ocean acidification. The first hypothesis of enhanced primary production under ocean acidification, due to higher CO_2 substrate availability, has been challenged in the last few years as more experiments have been performed. **It seems that the response of the plankton community to ocean acidification depends on the oceanic provinces, nutrient conditions, community composition and other unidentified processes (e.g. grazing).**

3.3 Combined effects of warming and acidification

Ocean acidification and ocean warming (OW) will occur concomitantly and the interactive effects of both factors must be taken into consideration. Only few studies have reported their combined effects on plankton community. In the Bering Sea, an increase in carbon fixation under warmer conditions irrespective of $p\text{CO}_2$ level with a shift toward smaller nanophytoplankton species was measured (Hare et al., 2007) and a similar experiment led to the same conclusions in the North Atlantic Ocean (Feng et al., 2009). Both experiments suggest a greater influence of temperature compared to $p\text{CO}_2$, with some interactive effects. An increase in carbon fixation under combined OA and OW was observed in mesocosm experiments (Kim et al., 2013) where, despite no change in the POC concentrations, enhanced DOC production at high temperature and/or CO_2 treatments (Kim et al., 2011) was measured. The bacterioplankton community structure has also been investigated under combined OA and OW using mesocosms (Lindh et al., 2013). OA alone had a limited impact, while temperature was the major driver causing shifts in species composition and synergistic effect of both factors might increase the species selection, highlighting the need to perform studies combining the two stressors. **There is a distinct lack of studies on the combined effect of OA and OW, despite it is important to know the possible interactions between these stressors and to know which driver could have the greatest impact on the plankton community in order to have realistic projections models.**

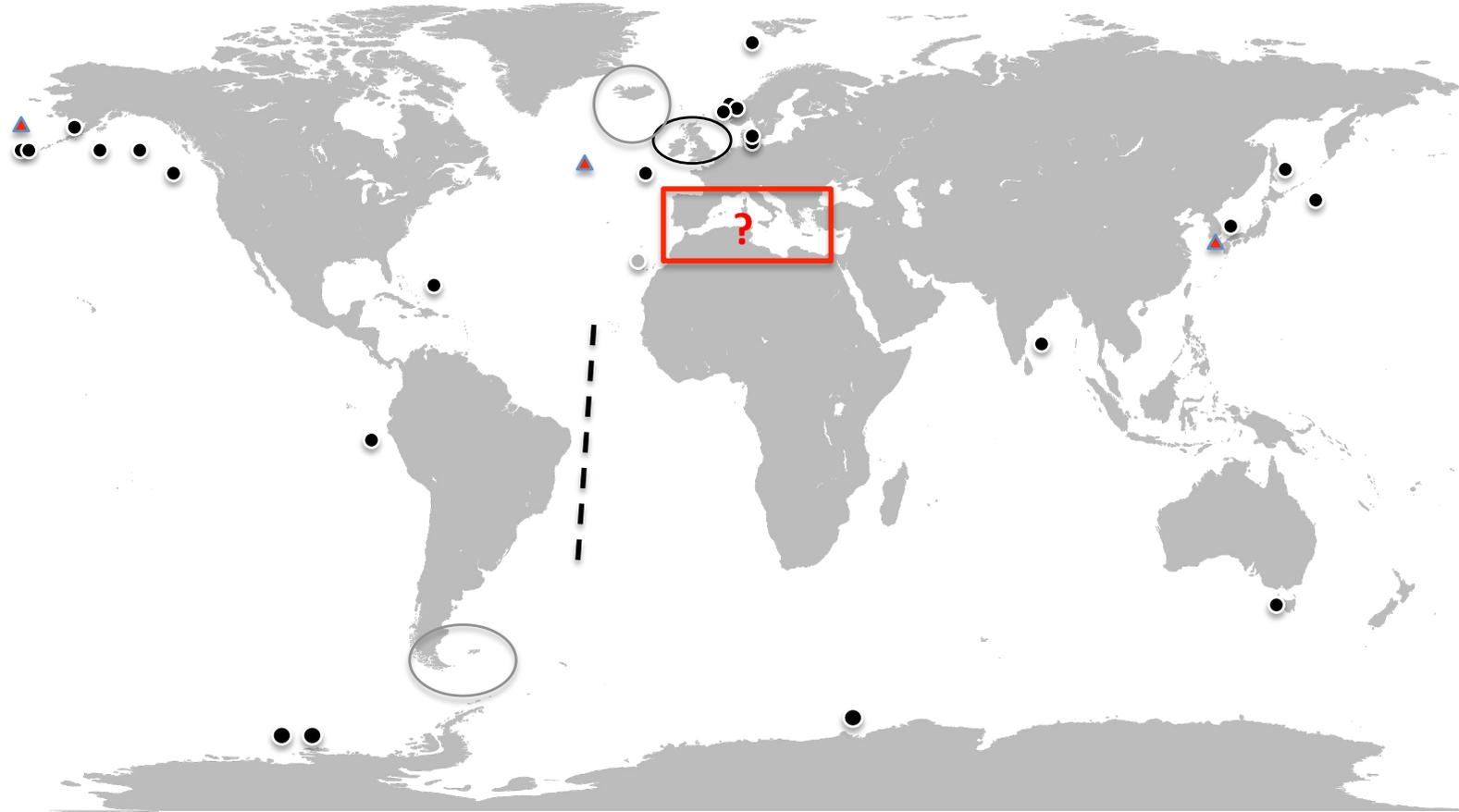


Figure I-5. Map showing locations of experiments on ocean acidification (OA; black points) or ocean acidification and warming (OA + OW; red triangles) effects on the plankton community undertaken from 1990 to 2014. The black line represents transect from Hein and Sand-Jensen (1997). The grey point offshore the north-west African coast was an experiment performed in January 2014 in Canary Islands which will be rerun in winter 2014 by KOSMOS team (Kiel, Germany). The grey circles in the Arctic and Antarctic are the cruises of the Sea Surface consortium UKOA performed in 2011 and 2012 that are currently unpublished. The black circle is also from the UKOA cruise of June-July 2011, which will be published in a special issue of Biogeosciences. The black point in the Bermuda area was a study with a community dominated by cyanobacteria (*Synechococcus* and *Prochlorococcus*). Map source: Wikimedia commons.

4. Oligotrophic areas under anthropogenic perturbation

Most of the experiments discussed in the previous section were performed in relatively eutrophic conditions or with nutrient addition during the experiment and were mainly carried out in relatively cold waters (Figure I-5). 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 its surface being associated with low productivity, termed oligotrophic areas. **A decreased nutrient availability and expansion of low productive regions are projected in a high CO₂ world, as enhanced thermal stratification is expected to lead to surface layer nutrient depletion (Polovina et al., 2008; Irwin and Oliver, 2009).**

Although it is important to assess the capacity of oligotrophic provinces for carbon uptake, and further storage, and its evolution under climate change, their trophic state (i.e. auto- vs heterotrophy) is still under debate (Ducklow and Doney, 2013), as to whether these areas are autotrophic (Williams et al., 2013) or heterotrophic (Duarte et al., 2013). As reviewed in the previous section, the effects of OA and/or OW on metabolic processes are still poorly understood and the biological response to climate change seems to be conditioned by the ecosystem conditions, e.g. nutrient availability, community composition. Therefore, the different biological responses in different oceanic regions must be investigated in order to gain a better understanding on the response of the global ocean to future environmental conditions. **However, oligotrophic areas have been chronically under sampled with respect to the effect of climate change.**

Case study: The Mediterranean Sea

The Mediterranean Sea (MS) is considered as an oligotrophic area exhibiting a gradient from mesotrophic (western basin) to ultra-oligotrophic (eastern basin). It is semi-enclosed, warm, deep and presents higher salinity and total alkalinity levels than in the open ocean. Mediterranean waters can, therefore, absorb more CO₂ than the open ocean waters. The western and eastern basins differ in their carbonate chemistry; the western basin exhibits a lower total alkalinity than the eastern basin and the opposite pattern is seen for C_T (higher C_T in the western than in the eastern basin). Based on satellite observations, it is estimated that the MS, as a whole, acts as a small sink of CO₂ (0.24 Gt C yr⁻¹), with the western basin acting

as a sink ($8.94 \text{ Gt C yr}^{-1}$) and the eastern basin as a source (8.4 Gt C yr^{-1} ; D'Ortenzio et al., 2008).

It has been suggested that the MS shifted from a source of CO_2 ($0.62 \text{ Gt C yr}^{-1}$) in 1960 to a sink ($-1.98 \text{ Gt C yr}^{-1}$) in 1990, but this was not accompanied by a significant decrease in pH using a surface layer box model couple with datasets available (Louanchi et al., 2009). However using data collected at the DYFAMED site, changes in surface water carbonate chemistry in the western basin were estimated and these suggest that pH has decreased by 0.15 pH units since the industrial revolution (Touratier and Goyet, 2011). It has been predicted that a decrease by another 0.3 to 0.4 pH units will occur for the end of the century in the Northwestern MS (Geri et al., 2014). From time series (1975-2004) located in the NW Mediterranean sea it has been estimated that temperature increased during this period with a rate of 0.026 to $0.033 \text{ }^\circ\text{C yr}^{-1}$ (Bensoussan et al. 2009). Using satellite observations it has been estimated that surface temperature in the MS has increased by 0.03 to $0.05 \text{ }^\circ\text{C yr}^{-1}$ in the western and eastern basins respectively corresponding to an increase of 0.66 and $1.1 \text{ }^\circ\text{C}$ over the time considered (1985-2006) and with noticeable seasonal variability (Nykjaer, 2009). This sea surface temperature increase rate was also found for the period 1982-2012 and CMIP5 projections predicted a further increase of $2.6 \text{ }^\circ\text{C}$ for 2100 for the worst case scenario (RCP 8.5; Shaltout and Omstedt, 2014), with significant seasonal and spatial variability.

Experiments on the effects of climate change on the Mediterranean plankton community are very scarce. On the western French coast, the mesocosm facilities of the Mediterranean platform for Marine Ecosystems Experimental Research (MEDIMEER) have set up a mesocosm facility in the Thau lagoon, that was used to carry out the first studies on the effect of environmental perturbation on plankton community. So far, the experiments mainly focused on the effects of increased nutrients, irradiance and temperature rather than ocean acidification effect. Vidussi et al. (2011) and Fouilland et al. (2013) showed that temperature had a greater effect than irradiance, increasing the abundance of ciliates and flagellates and decreasing the abundance of bacteria and copepods. The effects of the temperature increase on the community structure was accompanied by enhanced autotrophic processes that suggest a strengthening of the carbon pump under warmer conditions. These results cannot be extrapolated to the rest of the Mediterranean Sea because coastal lagoons have distinct environmental characteristics as well as different community compositions than open sea oligotrophic areas.

Currently, the best approximations on the effects of climate change on plankton community arise from time series of *in situ* and satellite observations, causing difficulty in

identifying which environmental parameter causes the biological modification. In the Bay of Calvi (Corsica, France), a decrease in biomass from 1979 to 1998 was detected (Goffart et al., 2002) and was associated to changes in nutrient concentrations resulting from reduced winter mixing. A shift toward smaller species (picoplankton and nanoflagellates) and a decline of diatoms, associated with more regenerated production and increased primary production per unit of chlorophyll, have been suggested and linked to increased cyanobacteria abundance at DYFAMED site in the Ligurian Sea (Marty and Chiavérini, 2002). Shifts in species assemblages and phenology, and decreased richness of the dinoflagellate *Ceratium*, have been linked to ocean warming based on time series and historical data (Tunin-Ley et al., 2009).

To date, and to the best of our knowledge, there has not been any reported experiments on the combined effects of ocean acidification and/or warming on the Mediterranean plankton community, despite the fact that the MS reacts rapidly to external perturbations.

5. Objectives and experimental approaches followed in this thesis

This work investigates the effects of ocean acidification and warming on the plankton community of the Northwestern Mediterranean Sea focusing on several major questions:

- What is the effect of ocean acidification on the metabolic rates of a plankton community maintained in close-to-natural conditions?
- Which groups benefit or are negatively affected by ocean acidification?
- What is the effect of ocean acidification and warming on plankton community structure and functioning?

To assess these questions, different approaches have been used in terms of experimental set-up (bottle incubations vs. mesocosms) and metabolic rate measurement methods (O₂ light-dark, ¹⁴C and ¹⁸O labelling). Moreover, a relatively novel approach based of ¹³C labelling coupled with biomarker analyses has been used in order to trace carbon flow between the different compartments of the community and to estimate specific-carbon fixation rates.

Outline of the thesis

The chapters correspond to the different questions and approaches used (Figure I-6). **Chapter II** discusses the effects of ocean acidification on metabolic rates measured using different methods during two mesocosm experiments performed in the NW Mediterranean Sea. During the same experiments, a ^{13}C labelling study was undertaken to investigate group-specific responses to ocean acidification, the results of which are reported in **chapter III**. In **chapter IV**, the effects of ocean acidification and warming on a post-bloom community were studied in smaller volumes. **Chapter V** synthesizes the results of all three studies and discusses them in a more general context.

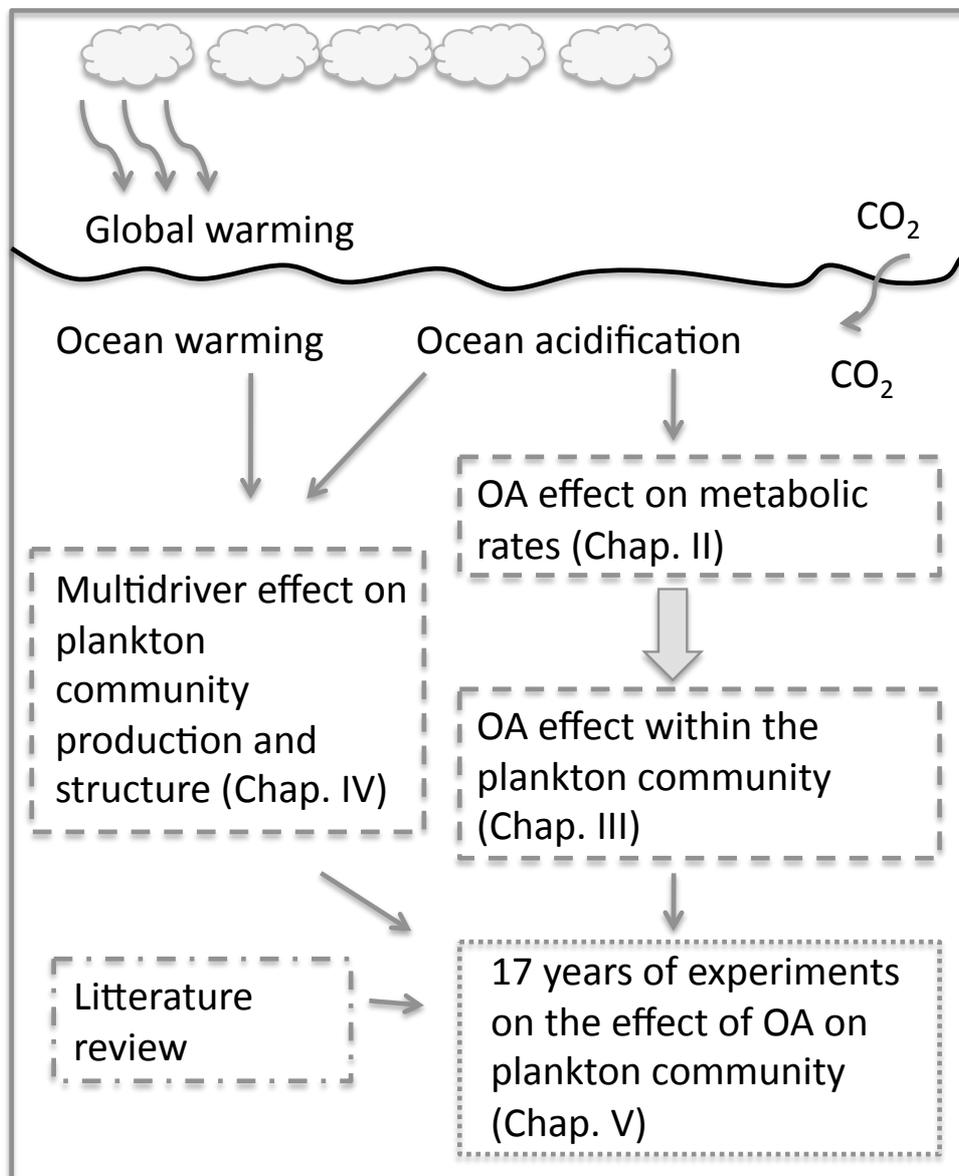


Figure I-6. Diagram presenting the objective of the thesis.

Chapter II

Ocean acidification and plankton metabolism in LNLC areas

1. Context of mesocosm experiments

In the frame of the MedSeA project (7th framework European project; <http://medsea-project.eu>), two mesocosm experiments were performed in the Northwestern Mediterranean Sea. These experiments were coordinated by the “Laboratoire d’Océanographie de Villefranche” (LOV-UMR 7093) in June-July 2012 in the Bay of Calvi (STARESO station, Corsica, France) and in February-March 2013 in the Bay of Villefranche (LOV, France). The mesocosm facilities were developed at LOV in the frame of the DUNE project (<http://www.obs-vlfr.fr/LOV/DUNE/index.html>) and were designed to avoid any contamination (e.g. metals) from the structures (Guieu et al., 2010).

The mesocosm set-up and general conditions will be fully described in Gazeau et al. (in prep, a). However, for clarity, we will briefly provide here some informations on the experimental set-up, study sites, as well as main results concerning hydrological conditions (temperature and salinity), carbonate chemistry and pigments. These data will introduce the results on plankton metabolism (Chapter II.2) and stable isotope analysis coupled with biomarkers (Chapter III.2).

1.1 Mesocosms acidification and sampling

For both experiments, a $p\text{CO}_2$ gradient approach has been chosen with 3 controls and 6 mesocosms with increasing $p\text{CO}_2$ levels. The gradient approach, has been preferred to the replicated ANOVA approach because of the restricted number of mesocosms and the high probability to lose one of the replicates making statistical analyses impossible. Furthermore, the gradient approach allows the study of the community response to various $p\text{CO}_2$ levels in order to evaluate any non-linear impacts and potentially identify tipping points (Barry et al., 2010).

To acidify the mesocosms, seawater was pumped, sieved through 5 mm to remove large organisms, and stored in a clean tank for acidification by bubbling CO_2 gas until complete saturation ($\text{pH} < 5$). A determined volume of CO_2 saturated seawater was then added to each “high- CO_2 perturbation” mesocosm over the 12 m depth with a diffusing system. The volume to add was determined using the “pmix” function of the Seacarb R package (Lavigne et al., 2014). The pH of the “perturbation” mesocosms was gradually decreased over a period of 4 days. The mesocosms were referred to as C1 to C3 for the

controls and P1 to P6 for the “perturbation” mesocosms, with increasing $p\text{CO}_2$ levels. At both sites, three groups (clusters) of three mesocosms were installed and each cluster was composed of a control, medium and high CO_2 level mesocosm (cluster K1: C1, P1, P4; cluster K2: C2, P2, P5; cluster K3: C3, P3 and P6; Figure II-1).

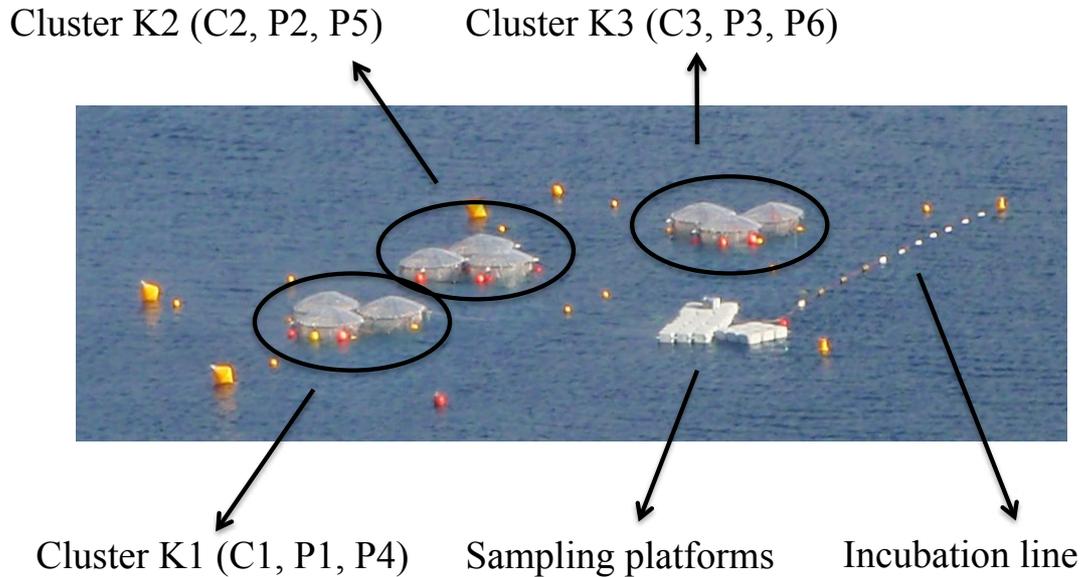


Figure II-1. Mesocosms deployed in the Bay of Calvi showing the different clusters and mesocosms (see text for more details). The platforms used to sample the mesocosms are also visible. Incubations for processes measurements (metabolic rates, nitrogen fixation, etc...) were suspended below the incubation line at the depth of mean irradiance in the mesocosms (~6 m in the Bay of Calvi and ~4 m in the Bay of Villefranche).

The sampling in the mesocosms started at the end of the acidification period (referred to as d0). Three, integrated 5 L water-samplers (Hydro-Bios) were used (one by cluster) to sample the water column from 0 to 10 m in each mesocosm, as well as outside the mesocosms (OUT) for some parameters. The sampled water was then transferred to subsampling bottles, specific to each measured parameter/process and brought to the laboratory. Samples for core parameters were taken on a daily basis at 8:30 am (local time). Every 2 days, additional parameters were sampled at 10:00 am and samples for metabolic process determination (e.g. nitrogen fixation, O_2 metabolism, bacterial production, etc) were taken every other day before sunrise, i.e. 4:00 am in the Bay of Calvi (BC) and 5:00 am in the Bay of Villefranche (BV). CTD, fluorometry, dissolved oxygen and PAR profiles from 0 to 10 m were performed daily (at midday in BC and at 10:00 am in BV), by means of a SBE19plusv2. Following the protocol of Czerny et al. (2013a), N_2O gas was added in one mesocosm to estimate air-sea gas

transfer velocities, under the assumption that this gas is not influenced by biological activity. Mesocosm volume was estimated, for calculation of the elemental budgets by adding a known amount of seawater saturated with sodium chloride (NaCl) (Czerny et al., 2013b).

Pigment concentrations were used with CHEMTAX for determination of phytoplankton groups that are reported in the next sections. This analysis was performed using an input matrix adapted to the Mediterranean Sea (Rodriguez et al., 2006; Not et al., 2007). The carbonate chemistry was calculated using Seacarb package based on measurement of alkalinity (A_T), dissolved inorganic carbon (C_T), salinity and temperature. The other parameters reported here, are from personal communications: J. Louis (LOV, France) for nutrient levels, P. Pitta (HCMR, Greece) for flow cytometry data in the Bay of Calvi and from M.-L. Pedrotti (LOV, France) in the Bay of Villefranche, and V. Taillandier (LOV, France) for CTD data. The following section reports solely on parameters that are relevant for understanding the results of this thesis (i.e. metabolic rates and stable isotopes coupled with biomarkers) and interpretation of these results is based on discussion with other scientists involved in the experiments.

1.2 Main results of Corsica mesocosm experiment

The Corsican coast is isolated from more productive waters at the centre of the Ligurian Sea, due to the uplift of the Ligurian Current along the coastal area (Figure II-2). The Bay of Calvi (NW Corsica, France; Figure II-2) was chosen as being typical of the oligotrophic conditions of low nutrient and low chlorophyll (LNLC) levels. Furthermore, the site “Pointe de la Revelatta”, where mesocosms were moored, is a protected area (<http://www.conservatoire-du-littoral.fr>) with restricted car access, few constructions and no river discharge. All these conditions make the site characteristic of clean oligotrophic areas, representative of offshore waters.

In June and July 2012, the construction and installation of the nine mesocosms took place from June 4th to 19th. The acidification period took 4 days and sampling started on June 24th. The experiment lasted 22 days (until July 16th). However, due to high wind speeds, sampling could not be done at the end of the experiment (June 15th) and the last sampling day considered for data analysis is July 14th (i.e. 20 day experimental period).

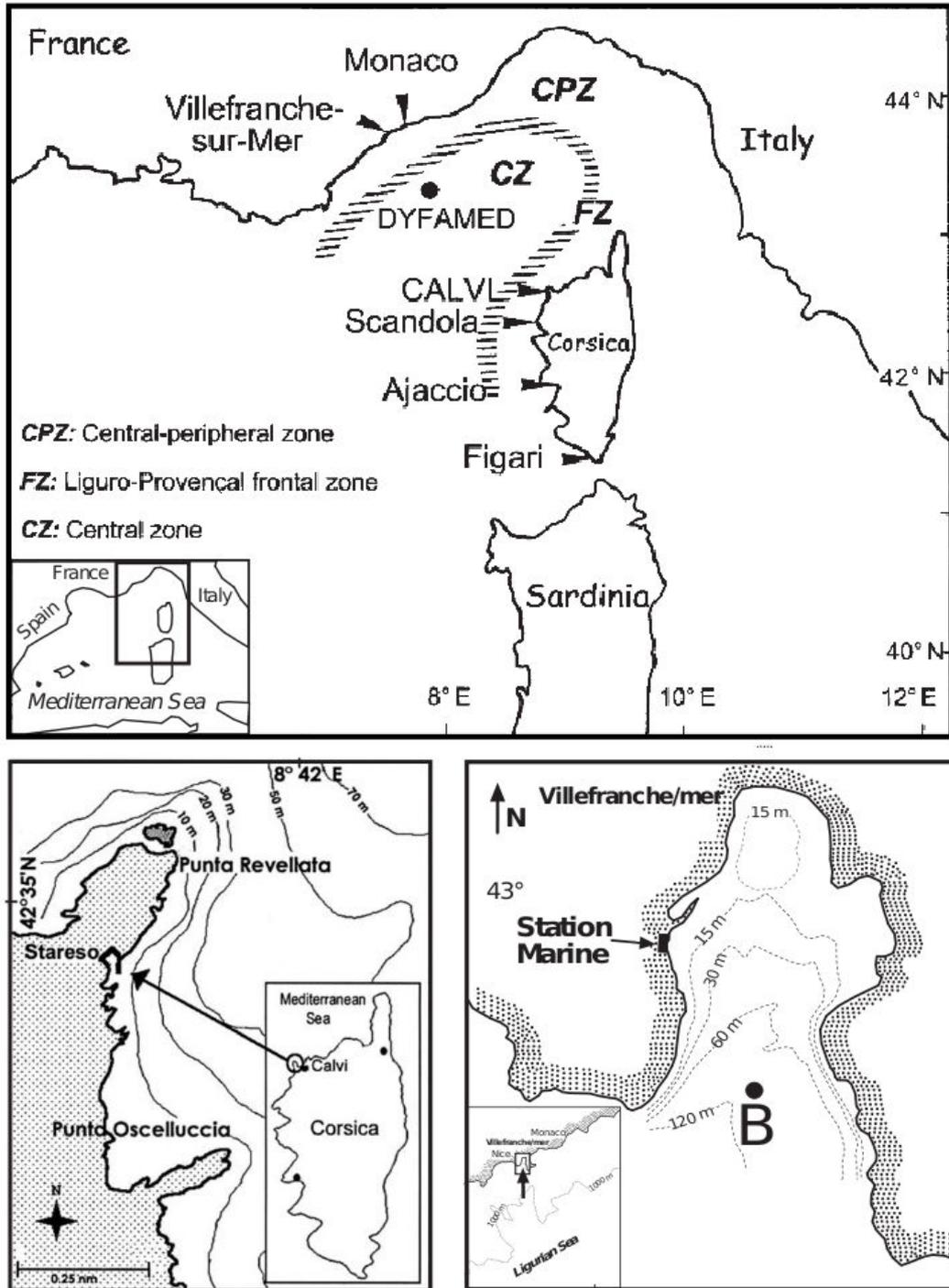


Figure II-2. Localisation of the study sites. Upper map: general view of the area with hydrological features. Lower left map: the Bay of Calvi and localisation of Stareso marine station, mesocosms were moored in front of the station by 20 m depth. Lower right map: the Bay of Villefranche with localisation of the Laboratoire d'Océanographie de Villefranche (LOV) and Point B time series, mesocosms were moored at 20 m depth in front of LOV. Source: upper panel from Goffart et al. (2002); lower left from Jadot et al. (2002); lower right from Gomez and Gorsky (1998).

The pH and $p\text{CO}_2$ evolutions over the experimental period are presented in Figure II-3. The natural decrease in $p\text{CO}_2$ in the high- CO_2 mesocosms was due to sea-air exchange. The different mesocosms were, from the beginning to the end of the experiment, clearly separated in terms of pH, except P4, P5 and P6 at the end of the experiment that tended to be similar in term of pH and $p\text{CO}_2$. Initial and final dissolved inorganic carbon (C_T) and alkalinity (A_T) concentrations are presented in Table II-1. As expected, A_T did not differ between mesocosms but increased over time by ca. $20 \mu\text{mol kg}^{-1}$, following the general, increasing trend of salinity. At the end of the experiment, salinity was higher inside than outside the mesocosms due to evaporation. Temperature increased over the experiment, similarly to conditions outside the mesocosms (from $21.5 \text{ }^\circ\text{C}$ to $24.2 \text{ }^\circ\text{C}$). CTD profiles show a homogeneous water column most of the days, except from days 5 to 8 where a thermal stratification occurred in all mesocosms and was also observed in the bay (Figure II-4a).

Tableau II-1. Initial and final concentrations of dissolved inorganic carbon (C_T) and total alkalinity (A_T) in $\mu\text{mol kg}^{-1}$ as mean integrated value over the 10 m depth in the nine mesocosms deployed in the Bay of Calvi in June/July 2012 (see text for more details). Concentrations measured in the bay (outside mesocosms; OUT) are also presented.

	OUT	C1	C2	C3	P1	P2	P3	P4	P5	P6
C_T										
initial	2225	2231	2227	2224	2282	2320	2335	2364	2408	2428
final	2232	2220	2229	2226	2261	2285	2304	2330	2326	2329
A_T										
initial	2532	2529	2530	2428	2527	2529	2529	2530	2529	2530
final	2544	2548	2549	2544	2550	2549	2552	2548	2446	2554

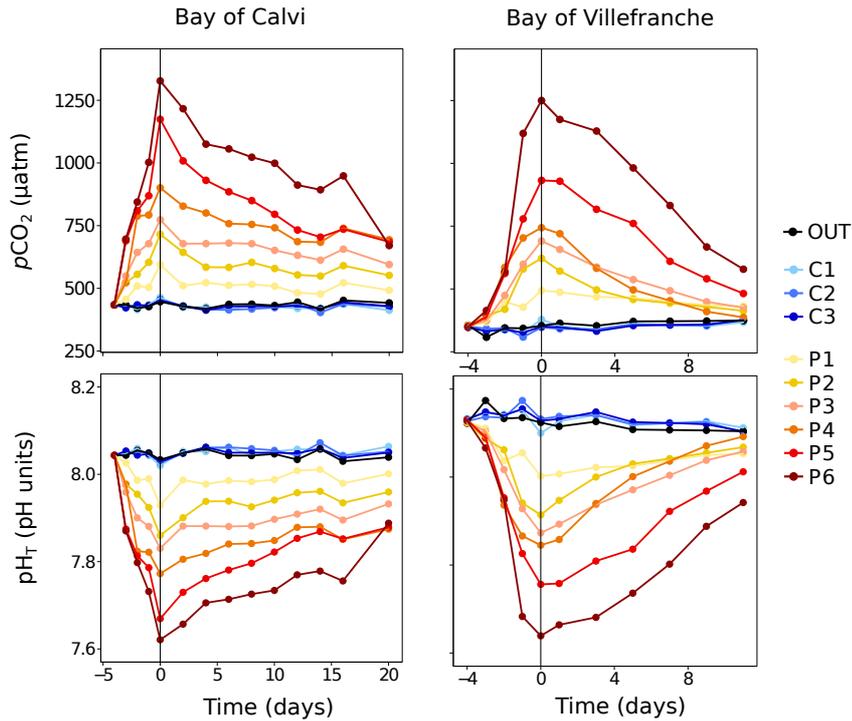


Figure II-3. Partial pressure of CO₂ ($p\text{CO}_2$; upper panel) and pH on the total scale (pH_T ; lower panel) inside and outside the mesocosms deployed in the Bay of Calvi (left) and the Bay of Villefranche (right).

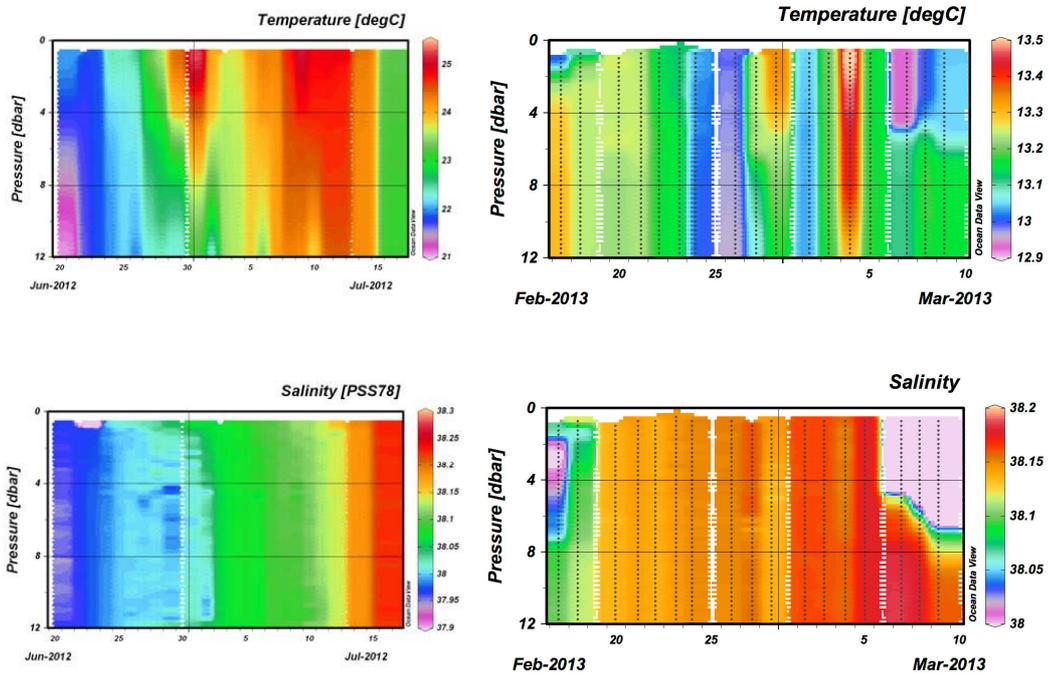


Figure II-4. Temperature (top) and salinity (bottom) profiles in the Bay of Calvi (BC; left) and Villefranche (BV; right) in mesocosm C1. In BC, thermal stratification is clearly visible on days 5 to 8. In BV, the intrusion of seawater from outside at the end of the experiment is clearly visible in both salinity and temperature profiles.

Initial, *in situ*, nutrient, particulate organic matter and chlorophyll *a* concentrations were low, as expected for this summer period (Table II-2). Nitrogen ($\text{NO}_x = \text{NO}_3^- + \text{NO}_2^-$) and phosphate (DIP) were always below 150 and 26 nmol L^{-1} , respectively and were similar inside and outside the mesocosms.

Chlorophyll *a* in the bay varied from 0.10 to 0.19 $\mu\text{g L}^{-1}$ (Figure II-5) during the course of the experiment. Haptophytes dominated the phytoplankton community with biomass around 0.04 $\mu\text{g L}^{-1}$, followed by cyanobacteria, chlorophytes and pelagophytes. Then, dinoflagellates and praeasinophytes were detected with concentrations below 0.01 $\mu\text{g L}^{-1}$ and diatoms were quasi absent in the bay.

Inside the mesocosms, during the course of the experiment chlorophyll *a* showed an increasing trend from d0 to d10 with very low concentrations from 0.06 to 0.09 $\mu\text{g L}^{-1}$ (Figure II-5). The most abundant phytoplankton groups were haptophytes and cyanobacteria. During the first days of the experiments, haptophytes tended to decrease in biomass while cyanobacteria increased and after d6, concentrations were relatively constant for both populations at levels of about 0.018 ± 0.004 and 0.018 ± 0.003 $\mu\text{g L}^{-1}$, respectively. *Synechococcus* (cyanobacteria) abundance (determined by flow-cytometry) did also show an increase from d0 to d10 and then decreased. Chlorophytes increased during all the experiment from 0.010 to 0.020 $\mu\text{g L}^{-1}$, their biomass being as important as cyanobacteria biomass at the end of the experiment. Pelagophytes, dinoflagellates and diatoms were then the most important populations in terms of biomass with concentrations around 0.005 $\mu\text{g L}^{-1}$. While dinoflagellates and diatom concentrations were relatively constant throughout the experiment, pelagophytes presented a peak on d14 (biomass of 0.014 ± 0.004 $\mu\text{g L}^{-1}$), a peak that is also visible on chlorophyll *a* values (Figure II-5). Bacteria and virus abundances were constant (data not shown).

Tableau II-2. *In situ* concentrations of nitrate + nitrite (NO_x) and phosphate (DIP), silicate (Si), ammonium (NH₄⁺), particulate organic nitrogen and carbon (PON and POC) and chlorophyll *a* (chl *a*) when sampling started (d0; June 24th in the Bay of Calvi and February 21st in the Bay of Villefranche. When data at d0 were not available, values have been measured on February 22nd (d1; *).

	NO _x	DIP	Si	NH ₄ ⁺	PON	POC	chl <i>a</i>
	(nmol L ⁻¹)			(μmol L ⁻¹)		(μg chl <i>a</i> L ⁻¹)	
Bay of Calvi	49.8	34.8	1.9	0.15	0.58	5.5	0.12
Bay of Villefranche	1166*	10.3	1.4*	0.06*	0.81*	7.96*	0.95

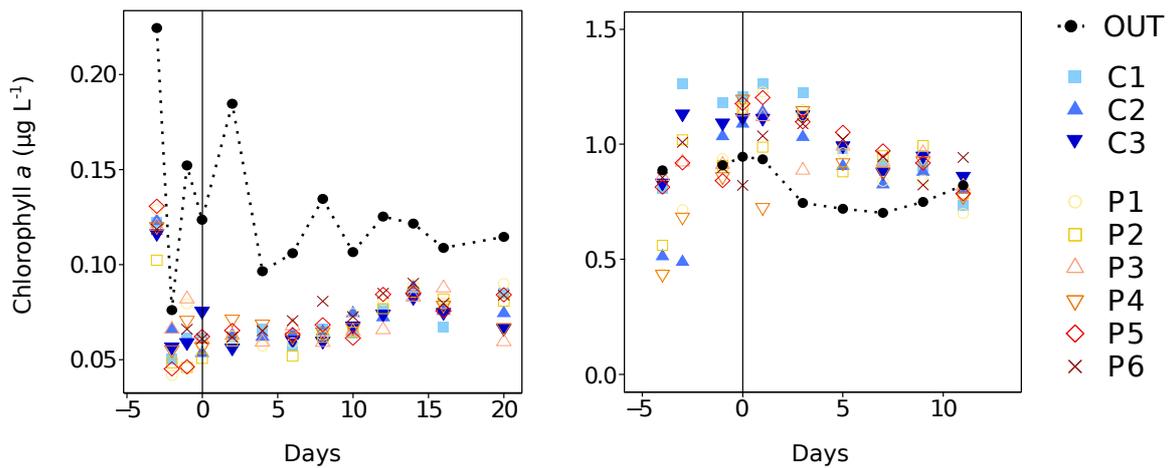


Figure II-5. Chlorophyll *a* concentrations during the experiments in the Bay of Calvi (left) and the Bay of Villefranche (right). Data before sampling started (day 0) have been provided by CTD profiles, while high performance liquid chromatography started on day 0. Colours and shapes represent the different mesocosms.

1.3 Main results of Villefranche mesocosm experiment

The objective of the second experiment was to follow the response of a plankton community to ocean acidification, during the bloom period (mesotrophic conditions) in order to compare with results obtained in the Bay of Calvi during the oligotrophic summer period. Based on the long-term (> 10 years) analysis of chlorophyll *a* dynamics in the Bay of Villefranche, the period February to March was identified as the period presenting the highest probability of capturing a bloom. Therefore, the construction and installation of the nine mesocosms took place from February 4th to 15th in front of the laboratory (Figure II-2). The acidification period took 4 days and sampling started on February 21st for 16 days (until March 9th). However, during two days (March 6th and 7th) there was a strong windstorm and a large swell that broke the bags. The openings in the bags were not immediately identified but the vertical CTD salinity profiles clearly show outside seawater intrusion, (Figure II-4b) thus the data can only be used until March 5th (12 days). As the experiment ended, chlorophyll increased in the bay and it has been decided to continue collecting samples for core parameters and primary production to acquire an interesting set of high-frequency data in the Bay of Villefranche during a bloom period. This dataset and its interpretation are provided in Appendix A.

The pH and $p\text{CO}_2$ evolution over 12 days are presented in Figure II-3. The decrease in $p\text{CO}_2$ in the high- CO_2 mesocosms was much sharper than during the Corsica experiment; P4 rapidly joined the P3 level and at day 3 the P4 level was below P3. Wind speeds and, consequently, air-sea gas exchange velocities were much higher than during the Corsica experiment (one order of magnitude higher), explaining part of the rapid decrease. In addition, the plankton community was mostly autotrophic during the experiment and acted as a sink of CO_2 . Initial and final C_T and A_T concentrations are presented in Table II-3. A_T did not differ between mesocosms but increased over time by $15 \mu\text{mol kg}^{-1}$. Temperature varied around 13 ± 0.5 °C over the course of the experiment and was similar to outside levels. CTD profiles showed a homogeneous water column, except at the end of the experiment when external low saline water entered the bags (Figure II-4b).

Table II-3. Initial and final concentrations of dissolved inorganic carbon (C_T) and total alkalinity (AT) in $\mu\text{mol kg}^{-1}$ as mean integrated value over the 10 m depth in the nine mesocosms deployed in the Bay of Villefranche in February/March 2013 (see text for more details). Concentrations measured in the bay (outside mesocosms; OUT) are also presented. * measured on March 4th.

	OUT	C1	C2	C3	P1	P2	P3	P4	P5	P6
C_T										
initial	2269	2285	2269	2270	2341	2385	2401	2418	2453	2498
final	2293	2293	2273	2287	2317	2305*	2324	2313	2339	2310*
A_T										
initial	2557	2559	2561	2561	2561	2562	2560	2565	2563	2564
final	2560	2562	2561	2562	2565	2563*	2562	2556	2556	2562*

Initial *in-situ* nutrient, particulate organic matter and chlorophyll *a* concentrations are presented in Table II-2. DIP concentrations were always below 20 nmol L^{-1} inside mesocosms, similar to outside conditions, except for d6 when DIP in the bay reached 22 nmol L^{-1} , while in the mesocosms, DIP remained at ca. 10 nmol L^{-1} . During the acidification period, NO_x decreased, leading to concentrations below $0.5 \mu\text{mol L}^{-1}$ inside the mesocosm while, in the bay, concentrations were ca. $1.2 \mu\text{mol L}^{-1}$.

Despite this difference in nutrient availability, chl *a* concentrations were the same inside and outside the mesocosms. Chlorophyll *a* data from the time series of the Bay of Villefranche in 2013 (Point B) showed that our experiment took place between a pulse of Chl *a* and the bloom (data not shown). Diatoms, prasinophytes, pelagophytes, haptophytes and cryptophytes were dominant in the bay, with relatively similar concentrations, between 0.10 and $0.15 \mu\text{g L}^{-1}$, which increased from d5 to the end of the experiment. When the experiment ended, these populations continued to increase to a final concentration of $0.25 \mu\text{g L}^{-1}$. Another group composed of dinoflagellates, chlorophytes and cyanobacteria was constant in terms of biomass at around $0.05 \mu\text{g L}^{-1}$.

After closing the bags, the plankton community rapidly consumed nutrients initially present in the mesocosms, which led to a different community structure. When sampling started (d0), chl *a* concentrations in the mesocosms were, on average, $1.1 \pm 0.1 \mu\text{g L}^{-1}$ and decreased to $0.80 \pm 0.07 \mu\text{g L}^{-1}$ at the end of the experiment. The most important species in terms of biomass were haptophytes, which increased during the acidification period until d3 to reach a maximum of ca. $0.37 \pm 0.05 \mu\text{g L}^{-1}$, and then decreased to the end of the

experiment to $0.28 \pm 0.03 \mu\text{g L}^{-1}$. Cryptophytes and pelagophytes were the second most important populations, presenting the same dynamics than haptophytes. Prasinophytes and diatoms showed opposite patterns, decreasing during the acidification period, until d4, and then increasing to the end of the experiment. The biomass for these species, varied from 0.05 to $0.15 \mu\text{g L}^{-1}$. Chlorophytes, cyanobacteria and dinoflagellates were the least important populations with concentrations below $0.05 \mu\text{g L}^{-1}$. Cyanobacteria (based on pigments, as well as *Synechococcus* (cyanobacteria) abundance based on flow-cytometry) increased during the experiment. In all mesocosms, bacterial abundances increased over the experiment while virus abundance was constant (data not shown).

2. No effect of ocean acidification on planktonic metabolism in the NW oligotrophic Mediterranean Sea: results from two mesocosm studies

Note: This manuscript has been completed in July 2014 and will be submitted in the special issue on December 1st 2014. Some parts of the discussion might be modified, therefore please refer to the latest version of the article than the one available at the time of diffusion of the thesis manuscript.

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Abstract

Oligotrophic areas account for about 30% of oceanic primary production and are projected to expand in a warm high CO₂ world. Changes in primary production in these areas could have important impacts on future global carbon cycling. To assess the response of primary production and respiration of plankton communities to increasing partial pressure of CO₂ (*p*CO₂) levels in Low Nutrient Low Chlorophyll areas, two mesocosm experiments were conducted in the Bay of Calvi (Corsica, France) and in the Bay of Villefranche (France) in June-July 2012 and February-March 2013 under different trophic state, temperature and irradiance conditions. Nine mesocosms of 50 m³ were deployed for 20 and 12 days respectively, and were subjected to seven *p*CO₂ levels (3 control and 6 elevated levels). The metabolism of the community was studied using several methods based on *in situ* incubations (oxygen light-dark, ¹⁸O and ¹⁴C uptake). Increasing *p*CO₂ had no significant effect on gross primary production, net community production, particulate and dissolved carbon production, as well as on community respiration. These two mesocosms experiments, the first performed under low nutrient and low chlorophyll, suggest that in large areas of the ocean, increasing *p*CO₂ levels may not lead to a significant change of plankton metabolic rates and to sea surface biological carbon fixation.

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2.1 Introduction

Oceanic primary production represents about 50% of global primary production (Field et al., 1998) and plays a key role in climate regulation. The balance between the gross primary production (GPP) of autotrophic organisms and community respiration (CR) of both autotrophic and heterotrophic organisms determines the net community production (NCP), revealing the capacity of a system to sequester carbon via the biological pump. Production and consumption of organic matter depend on the composition of the plankton community and are constrained by environmental parameters such as nutrient availability (i.e. nitrogen, phosphorus, silicon concentration, ratios and chemical forms), light availability and temperature. The increase in the partial pressure of CO₂ ($p\text{CO}_2$) in the ocean and consequent decrease in seawater pH, so-called ocean acidification (Gattuso and Hansson, 2011), might also influence the metabolism of planktonic organisms and marine communities.

Many laboratory studies, focused on phytoplankton strains maintained in culture, have been performed to test the response of primary production to increased $p\text{CO}_2$, with contrasting results reported. While diatoms appear to generally benefit from an increase in CO₂, the response of coccolithophores is more variable: from increased production to neutral or even inhibitory effects under nitrogen limitation (see comprehensive review by Riebesell and Tortell, 2011). Phytoplankton use inorganic carbon to produce organic matter, however many species are limited by the low CO₂ affinity of the RuBisCO, an enzyme involved in CO₂ fixation (Raven and Johnston, 1991). To compensate for this low CO₂ affinity, some species have developed carbon concentrating mechanisms (CCMs; Giordano et al., 2005). As the efficiency of these CCMs can be highly variable between species, it is expected that organisms will be differently affected by CO₂ increases and shifts in the plankton community composition might therefore occur (Rost et al., 2008). Another drawback of single strain culture experiments is that the heterotrophic component of plankton communities is, for the most part, not taken into consideration. Yet, a possible indirect effect of elevated $p\text{CO}_2$ on bacteria has been suggested and linked to changes in phytoplankton activity (Grossart et al., 2006). Autotrophic organisms can indeed release dissolved organic carbon (DOC), which can in turn be used by bacteria for growth and respiration. An increase in DOC production under elevated $p\text{CO}_2$ could therefore have an impact on the bacterial community (see also Liu et al., 2010).

Experiments have recently been conducted to assess the effects of ocean acidification on natural plankton assemblages with results showing either increased photosynthesis and/or net community production with increasing $p\text{CO}_2$ (e.g., Riebesell et al., 2007; Egge et al., 2009) or

no effect (e.g., Hare et al., 2007; Tanaka et al., 2013). Some of these experiments at the community level have been conducted using pelagic mesocosms. This approach is considered to be closer to the “real world” because large mesocosms enclose a significant volume of seawater containing an entire plankton community with environmental conditions (e.g., temperature, irradiance, water motion) within the mesocosm similar to those prevailing *in situ* (Riebesell et al., 2010, 2013).

Most of the experiments have been performed in high-nutrient or nutrient-enriched systems and very few experiments have been reported in low nutrient areas (Yoshimura et al., 2010). Yet, pelagic primary production is highly variable between oceanic provinces and more than 60% of the open ocean is considered to be oligotrophic (i.e. low nutrient). Despite their low nutrient concentration and relatively low productivity, these areas represent about 30% of oceanic primary production (Longhurst et al., 1995). Furthermore, it has been suggested that oligotrophic areas will expand as a result of ocean warming (Polovina et al., 2008), with potential implications for ocean biogeochemistry and primary production (Irwin and Oliver, 2009). Although the metabolic status of open ocean waters is still hotly debated (Duarte et al., 2013; Williams et al., 2013), any change due to ocean acidification and/or warming will undoubtedly have profound impacts on the biological carbon pump and carbon cycle. The Mediterranean Sea, a semi-enclosed sea, is characterized by low nutrient and low chlorophyll (LNLC) concentrations, although depending on the location and season, trophic conditions can be defined as ranging from mesotrophic to ultra-oligotrophic (D’Ortenzio and d’Alcalà, 2009).

To test whether ocean acidification will affect plankton community composition and functioning in oligotrophic areas, two mesocosm experiments were performed in the Northwestern Mediterranean Sea during two contrasting periods (winter *vs.* summer), in the framework of the European Mediterranean Sea Acidification in a Changing Climate project (MedSeA; www.medsea-project.eu). Here, we report on the effects of ocean acidification on planktonic metabolism (gross primary production, net community production, particulate and dissolved carbon production as well as community respiration), as measured using several methods (the oxygen light-dark, ^{14}C and ^{18}O labelling techniques).

2.2 Material and Method

2.2.1. Study sites and experimental set-up

One mesocosm experiment was conducted in the Bay of Calvi (BC; Corsica, France) in summer (June-July 2012) and the other one in the Bay of Villefranche (BV; France) during the transition between winter and spring (February-March 2013). The experimental set-up and mesocosm characteristics are described in a companion paper (Gazeau et al., in prep, a). Briefly, for each experiment, nine mesocosms of ca. 50,000 L (2.3 m in diameter and 12 m deep) were deployed for 20 and 12 days in BC and BV, respectively. Once the bottom of the mesocosms was closed, CO₂ saturated seawater was added to obtain a *p*CO₂ gradient across mesocosms ranging from ambient levels to 1,200 µatm (see Table 2 and 3 for BC and BV, respectively), with three control mesocosms (C1, C2 and C3) and six mesocosms with increasing *p*CO₂ (P1 to P6). Measurements of planktonic metabolism started after the end of the CO₂ manipulation, on 24 June 2012 and 22 February 2013 for BC and BV, respectively. Before sunrise (04:00 in BC and 05:00 in BV; local times are used throughout this paper), depth-integrated sampling (0 to 10 m) was performed using 5 L Hydro-Bios integrated water samplers and distributed into various incubation bottles (see below). An incubation line was moored near the mesocosms and incubations took place at the depth of mean irradiance over the 12 m depth of the mesocosms (6 m for BC and 4 m for BV; see section on irradiance below for more details). During both experiments, net community production (NCP) and community respiration (CR) were determined using the oxygen light-dark method every two days. Gross primary production (GPP) was measured using the ¹⁸O-labelling method (GPP-¹⁸O) every 4 days during the BC experiment, while rates of particulate organic (PP-¹⁴C) and dissolved organic production (DO¹⁴Cp) were determined every two days using the ¹⁴C labelling technique during the BV experiment.

2.2.2. Irradiance

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

2.2.3. Oxygen light-dark method

From each mesocosm, 15 biological oxygen demand (BOD; 60 mL) bottles were filled, among which five were immediately fixed with Winkler reagents and used to estimate initial dissolved oxygen (O₂) concentrations. Five transparent bottles were incubated *in situ* on the incubation line for 24 h to estimate NCP. In order to estimate CR, 5 bottles were incubated for 24 to 36 h in the dark in a laboratory incubator at *in situ* temperature (ca. 23 °C for BC and ca. 13 °C for BV). Upon completion of the incubations, samples were fixed with Winkler reagents. O₂ concentrations were measured using an automated Winkler titration technique with potentiometric end-point detection. Analyses were performed with a Metrohm Titrand 888 and a redox electrode (Metrohm Ag electrode). Reagents and standardizations were similar to those described by Knap et al. (1996). NCP and CR were estimated by regressing O₂ values against time, and CR were expressed as negative values. Gross primary production (GPP-O₂) rates were calculated as the difference between NCP and CR. The combined errors were calculated as $S.E._{x-y} = \sqrt{(S.E._x^2 + S.E._y^2)}$.

2.2.4. GPP-¹⁸O method

In BC, every 4 days, water samples from each mesocosm were transferred into eight transparent glass bottles (60 mL) and sealed. Three bottles were immediately poisoned with 10 µL of a saturated mercury chloride (HgCl₂) solution in order to estimate the initial O₂ isotopic composition. The remaining five transparent glass bottles were spiked with 100 µL of 97% H₂¹⁸O in order to reach a δ¹⁸O-H₂O enrichment of 650‰ and were incubated *in situ* from sunrise to sunset. Upon completion of the incubation, samples were poisoned using 10 µL of HgCl₂, and stored upside down in the dark at room temperature pending analysis. Isotopic measurements were performed at Leuven University (Belgium). A headspace of 3 mL was created with helium and allowed to equilibrate for 30 min in order to measure ¹⁸O-O₂. The extracted water was then injected into helium-flushed vials for ¹⁸O-H₂O measurements. Pure CO₂ (100 µL) was then added and samples were allowed to equilibrate for 24 h. δ¹⁸O-H₂O was therefore measured as δ¹⁸O-CO₂. Determinations of δ¹⁸O-O₂ and δ¹⁸O-CO₂ were performed on an elemental analyzer (Flash HT/EA) coupled to a Delta V Isotope-ratio Mass Spectrometer (IRMS). An overflow technique was used to limit air contamination of the needle. For δ¹⁸O-O₂, the internal standard used to correct the data and monitor instrumental drift was air from the outside. For δ¹⁸O-CO₂, a calibration was performed against Vienna Standard Mean Ocean Water (VSMOW). GPP-¹⁸O rates (µmol O₂ L⁻¹ d⁻¹) were calculated using the following equation (Kiddon et al., 1995):

$$\text{GPP-}^{18}\text{O} = [(\delta^{18}\text{O-O}_{2\text{final}} - \delta^{18}\text{O-O}_{2\text{init}}) / (\delta^{18}\text{O-H}_2\text{O} - \delta^{18}\text{O-O}_{2\text{init}})] \times \text{O}_{2\text{init}}$$

where $\delta^{18}\text{O-O}_{2\text{init}}$ and $\delta^{18}\text{O-O}_{2\text{final}}$ are measured $\delta^{18}\text{O-O}_2$ before and after incubation (‰), $\delta^{18}\text{O-H}_2\text{O}$ is the final isotopic composition of the labelled seawater (‰), and $\text{O}_{2\text{init}}$ is the O_2 concentration before incubation ($\mu\text{mol O}_2 \text{ L}^{-1}$). The overall error was estimated using a Monte-Carlo procedure where one thousand values were randomly chosen between the mean \pm S.D. of each measured parameter and the mean \pm S.E. of each computed parameter is reported.

2.2.5. ^{14}C primary production

In BV, every 2 days, water samples from each mesocosm were transferred to four culturing flasks (40 mL) and spiked with 10 to 50 μCi of a ^{14}C -labelled sodium bicarbonate solution. Three flasks were incubated *in situ* for 24 h (sunrise to sunset). The remaining flask was immediately poisoned with 1 mL of a borax-buffered formaldehyde solution filtered through a 0.2 μm syringe tip filter and stored in the laboratory to estimate abiotic ^{14}C labelling. After 24 h, the samples were brought back to the laboratory and 3 mL was gently filtered through 0.2 μm polycarbonate filters directly into scintillation vials for DO^{14}Cp measurements (López-Sandoval et al., 2011). Scintillation vials were closed with a gas-tight rubber stopper and plastic centre wells containing a GF/A filter soaked with 200 μL of β -phenylethylamine. Then, 75 μL of hydrochloric acid (HCl; 50%) was injected into the vial in order to transform $^{14}\text{C-DIC}$ to $^{14}\text{CO}_2$, which was trapped by the β -phenylethylamine while ^{14}C -labelled DOC remained in the seawater.

The remaining 37 mL was then filtered through 0.4 μm polycarbonate filters (25 mm diameter) and rinsed with freshly filtered (0.7 μm) seawater. Filters were placed in scintillation vials that were closed with gas-tight rubber stopper and centre wells with a GF/A filter soaked with β -phenylethylamine, as for DO^{14}Cp . One mL of phosphoric acid (H_3PO_4 ; 1%) was injected through the rubber stopper onto the filter in order to dissolve ^{14}C -particulate inorganic carbon (Balch et al., 2000). After another 24 h, the centre wells and soaked GF/A filters were placed separately into fresh scintillation vials. Scintillation cocktail (15 mL; Ultima Gold MV, Perkin Elmer) was added to the vials containing the DOC (DO^{14}Cp) and the PC filter ($\text{PP-}^{14}\text{C}$) and activities were determined on a Packard Tri Carb (1600 CA) scintillation counter. Disintegrations per minute (DPM) were converted to production rates (after correction from abiotic ^{14}C labelling) using dissolved inorganic carbon concentrations measured in the mesocosm (Gazeau et al., in prep, a) and an isotopic discrimination factor of 1.05. In order to verify the initial spike activity, 100 μL of seawater from 3 to 6 random

culture flasks were removed and placed in a scintillation vial containing 200 μL of β -phenylethylamine and these were counted on the scintillation counter. The percentage of extracellular release (PER) was calculated as $\text{DO}^{14}\text{Cp}/(\text{PP-}^{14}\text{C} + \text{DO}^{14}\text{Cp})$ (López-Sandoval et al., 2011).

2.2.6. Data analysis, statistics and data availability

Results are reported as mean value \pm standard error (S.E.) as well as the average over all mesocosms \pm standard deviation (S.D.) when specified. Cumulative metabolic rates were calculated for the whole experimental period. Values for days when no incubations were performed were obtained by linear interpolation and the cumulative values were then summed up for the experimental period. The $p\text{CO}_2$ values used for the representation of cumulative metabolic rates are the average $p\text{CO}_2$ over the experimental period for each mesocosm. To test for $p\text{CO}_2$ increase effects, the relationship between cumulated metabolic rates and $p\text{CO}_2$ were realised using linear regressions. Linear regressions were also used to test for relationships between production rates with time and PAR, while Model-II linear correlation were used to compare metabolic rates obtained with the different measurements methods. All linear regression and correlation were performed using the R software (R Core Team, 2013) and were considered significant at a probability $p < 0.05$. The data sets are freely available on Pangaea, in the Bay of Calvi: <http://doi.pangaea.de/10.1594/PANGAEA.810331> and in the Bay of Villefranche: <http://doi.pangaea.de/10.1594/PANGAEA.835117>.

2.3 Results

2.3.1. Summer conditions (Bay of Calvi)

The initial temperature, salinity, and concentrations of nutrients and chlorophyll a inside and outside the mesocosms in the Bay of Calvi (BC) are shown in Table II-4 while the initial and mean $p\text{CO}_2$ values over the experiment are presented in Table II-5. Further details can be found in Gazeau et al. (in prep, a). At the start of the experiment (day 0), the concentration of nitrogen ($\text{NO}_x = \text{nitrate} + \text{nitrite}$) was similar inside and outside the mesocosms. In contrast, the concentrations of dissolved inorganic phosphate (DIP) and chlorophyll a were lower inside than outside the mesocosms (Table II-4).

NCP ranged from -2.7 ± 0.3 to $2.9 \pm 0.4 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ over the experimental period of 20 days (Figure II-6a). The lowest and highest values were measured in the control mesocosms, respectively C3 on day 16 and C1 on day 10. NCP was negative on day 0 and

tended to increase and reached a maximum value on day 8 or 10 (day 20 for P2), depending on the mesocosm. After this period of increase, NCP remained close to metabolic balance (ca. $0 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) until the end of the experiment. CR varied from -3.6 ± 0.2 to $0.2 \pm 0.4 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Figure II-6b). The lowest and highest values were measured in C2 on day 10 and P6 on day 2, respectively. Similar to NCP, the highest CR rates were measured on day 10 in all mesocosms apart from P2 for which the highest rate was measured on day 16 ($-2.63 \pm 0.20 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$).

Table II-4. Average environmental conditions at day 0 in all mesocosms (mean \pm S.D.): temperature, salinity as well as concentrations of nitrate + nitrite (NO_x), inorganic phosphate (DIP) and chlorophyll *a* (chl *a*). BC refers to the Bay of Calvi (Corsica, France) and BV to the Bay of Villefranche (France). * measured on February 19th.

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

GPP- O_2 ranged from -0.7 ± 1.1 to $5.5 \pm 0.5 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Figure II-6c). The lowest and highest rates were both measured in C1, on day 12 and day 10, respectively. After a stable period from day 0 to day 6, GPP- O_2 increased to reach a maximum value on day 10 for all mesocosms except C3 and P4, for which maximum values were reached on day 14 and day 8, respectively. GPP- ^{18}O varied from 0.0 ± 0.1 to $1.7 \pm 0.1 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Table II-5). The lowest value was measured in C2 on day 0, while the highest value was measured in P4 on day 16. GPP- ^{18}O was relatively stable during the experiment, showing a slight increase until day 16 and a decrease on day 20 (except for C2, which decreased from day 12). GPP- ^{18}O rates were generally lower than GPP- O_2 , with no significant correlation ($r^2 < 0.01$, $p > 0.05$, $n = 52$).

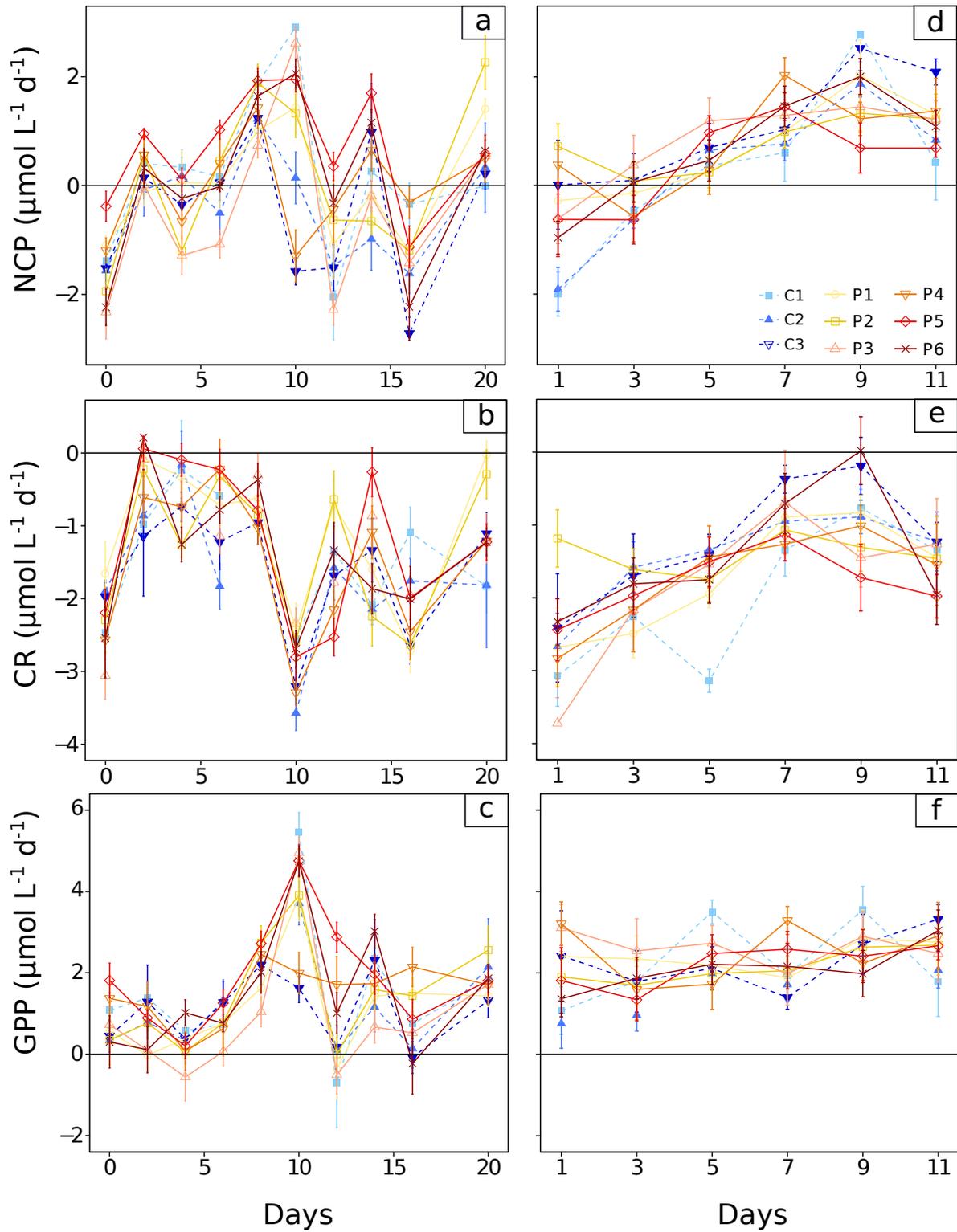


Figure II-6. Net community production (NCP; a and d), community respiration (CR; b and e) and gross primary production (GPP- O_2 ; c and f) as a function of time during the experiment in the Bay of Calvi (left) and in the Bay of Villefranche (right).

Table II-5. Gross primary production estimated by the ^{18}O labelling technique (GPP- ^{18}O ; $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) in the Bay of Calvi. Mean rates and associated standard errors (S.E.) are reported. For each mesocosm, the $p\text{CO}_2$ level on day 0 and averaged over the experimental period (20 days) are also reported (further details in Gazeau et al., in prep, a).

Mesocosm		C1	C2	C3	P1	P2	P3	P4	P5	P6
Initial $p\text{CO}_2$ (μatm)		463	455	452	595	716	774	901	1174	1327
Mean $p\text{CO}_2$ (μatm)		429	427	429	508	586	660	747	828	990
Day										
0	Mean	1.05	0.03	0.62	1.4	0.29	0.1	1	0.25	0.13
	S.D.	0.16	0.19	0.14	0.38	0.12	0.13	0.2	0.24	0.17
4	Mean	0.75	0.91	0.93	1.01	0.95	0.48	0.85	0.88	0.89
	S.D.	0.02	0.08	0.09	0.1	0.11	0.11	0.09	0.17	0.1
8	Mean	1.07	0.86	0.99	0.63	0.81	0.15	1.04	1.17	1.29
	S.D.	0.11	0.13	0.1	0.11	0.04	0.13	0.04	0.11	0.09
12	Mean	1.28	1.14	1.44	0.85	1.52	1.41	1.5	0.96	1.21
	S.D.	0.06	0.09	0.16	0.05	0.08	0.13	0.07	0.15	0.07
16	Mean	1.12	0.28	1.36	1.36	1.53	1.02	1.68	1.42	1.23
	S.D.	0.14	0.06	0.05	0.15	0.1	0.09	0.13	0.07	0.1
20	Mean	0.98	0.48	0.77	0.63	1.04	0.76	0.94	0.76	0.91
	S.D.	0.1	0.09	0.07	0.06	0.05	0.15	0.12	0.11	0.05

Cumulative NCP average over all nine mesocosms was $-1 \pm 8 \mu\text{mol O}_2 \text{ L}^{-1}$ and varied between -11.9 ± 1.8 and $13.6 \pm 1.2 \mu\text{mol O}_2 \text{ L}^{-1}$ (Figure II-7a) depending on the mesocosm with the lowest rates measured in C2, C3 and P3 and the highest estimated in P5. There was no significant trend in cumulative NCP with increasing $p\text{CO}_2$ ($r = 0.44$, $p > 0.05$, $n = 9$). The average cumulative CR was $-29 \pm 4 \mu\text{mol O}_2 \text{ L}^{-1}$, with no significant trend with increasing $p\text{CO}_2$ ($r = 0.30$, $p > 0.05$, $n = 9$). The cumulative GPP- O_2 and GPP- ^{18}O were on average $28 \pm 6 \mu\text{mol L}^{-1}$ and $20 \pm 4 \mu\text{mol O}_2 \text{ L}^{-1}$, respectively. For both methods there was no significant trend with increasing $p\text{CO}_2$ ($r = 0.47$, $p > 0.05$, $n = 9$ and $r = 0.17$, $p > 0.05$, $n = 9$, respectively). Mean daily PAR at 6 m (Figure II-8) was constant through time ($r = 0.04$, $p > 0.05$, $n = 10$) varying from 180 and 330 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$. GPP- O_2 and GPP- ^{18}O ($r = 0.54$, $p < 0.05$, $n = 88$ and $r = 0.27$, $p < 0.05$, $n = 54$, respectively) and NCP ($r = 0.30$, $p < 0.05$, $n = 90$) were significantly related to the PAR at 6 m. For all the results reported here, when metabolic rates were normalised to chlorophyll *a* concentrations as a best proxy for phytoplankton biomass, the same results were obtained either on the effect of $p\text{CO}_2$ increase or relationship between methods and with PAR.

2.3.2. Winter-spring conditions (Bay of Villefranche)

The initial temperature, salinity, and concentrations of nutrients and chlorophyll *a* inside and outside the mesocosms in BV are shown in Table II-4 while initial and mean $p\text{CO}_2$ values over the experiment are presented in Table II-6. Further experimental details can be found in Gazeau et al. (in prep, a). When sampling started (day 0), NO_x concentrations were higher outside than inside the mesocosms (Table II-4) and remained higher outside the mesocosm during the experimental period (Louis et al., in prep). In contrast, DIP and chlorophyll *a* concentrations inside and outside the mesocosms were initially similar (Table II-4).

NCP varied from -2.0 ± 0.4 to $2.8 \pm 0.5 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Figure II-6d). The lowest and highest values were both in C1, on day 1 and on day 9, respectively. NCP generally increased ($r = 0.57, p < 0.05, n = 52$) throughout the experiment from negative (heterotrophic system) to positive values (autotrophic system). NCP was negative on day 1 for all mesocosms except C3, P2 and P4 whereas, on day 5, all mesocosms had positive NCP. CR ranged from -3.7 ± 0.4 to $0.02 \pm 0.47 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Figure II-6e). The lowest value was measured in P3 on day 1 and the highest in P6 on day 9. CR rates generally decreased with time ($r = 0.63, p < 0.05, n = 54$): the highest rates being measured on day 1 (overall mean: $-2.6 \pm 0.6 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) and the lowest rates being measured on day 9 (overall mean: $-0.9 \pm 0.6 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$). GPP- O_2 ranged from 0.8 ± 0.6 to $3.6 \pm 0.6 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Figure II-6f) with a slight increasing trend as a function of time ($r = 0.43, p < 0.05, n = 54$).

Cumulative NCP average over all nine mesocosms was $7.4 \pm 2.6 \mu\text{mol O}_2 \text{ L}^{-1}$ and varied from 3.7 ± 0.8 to $11.8 \pm 1.6 \mu\text{mol O}_2 \text{ L}^{-1}$ (Figure II-7b). Cumulative CR was on average $-17.3 \pm 2.8 \mu\text{mol O}_2 \text{ L}^{-1}$ and, as for cumulative NCP, minima and maxima were measured in control mesocosms (C1 and C3, respectively) while the average GPP- O_2 was $24.7 \pm 2.7 \mu\text{mol O}_2 \text{ L}^{-1}$. Cumulative NCP, CR and GPP- O_2 did not show any significant trend with increasing $p\text{CO}_2$ (NCP: $r = 0.06, p > 0.05, n = 9$; CR: $r = 0.04, p > 0.05, n = 9$ and GPP- O_2 : $r = 0.02, p > 0.05, n = 9$).

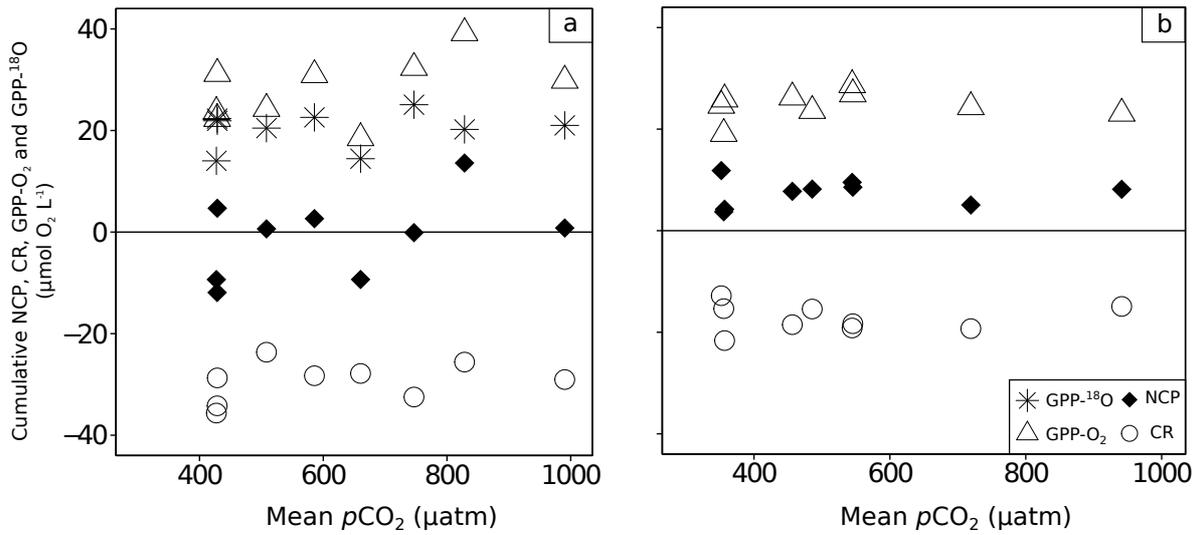


Figure II-7. Cumulative rates of net community production (NCP; full diamonds), community respiration (CR; empty circles) as well as gross primary production estimated using the oxygen light-dark (GPP- O_2 ; empty triangles) and the ^{18}O labelling (GPP- ^{18}O ; asterisk) techniques in the Bay of Calvi (a, duration: 20 days) and in the Bay of Villefranche (b, duration: 12 days). $p\text{CO}_2$ is the mean value for each mesocosm during the experiment.

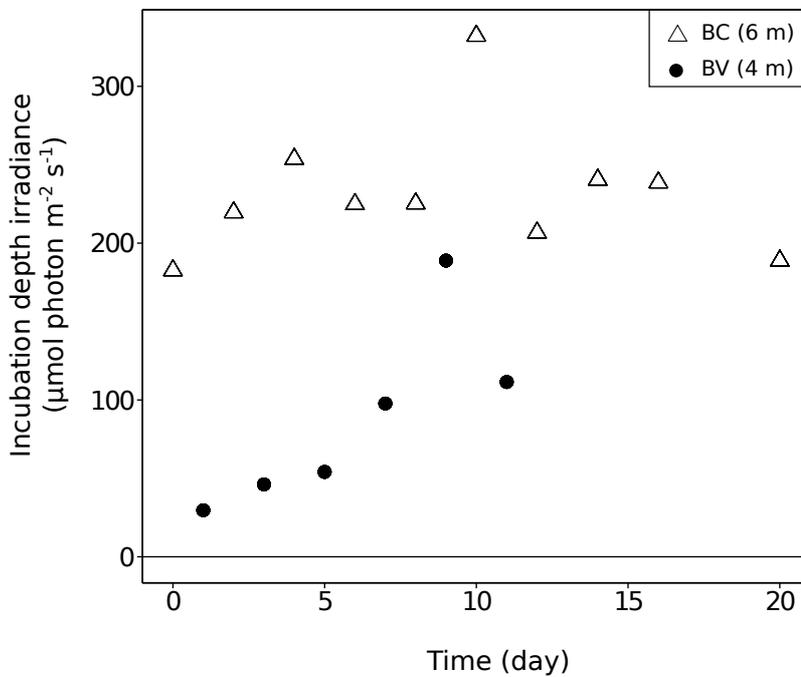


Figure II-8. Irradiance at the incubation depth in the Bay of Calvi (empty triangle) and in the Bay of Villefranche (circles) as a function of time.

Table II-6. Particulate and dissolved primary production (PP-¹⁴C and DO¹⁴C) during the experiment in the Bay of Villefranche. Rates are reported as mean value ± S.D. $\mu\text{mol C L}^{-1}$. For each mesocosm, the $p\text{CO}_2$ level on day 0 and averaged over the experimental period (12 days) are also reported (further details in Gazeau et al., in prep, a). NA indicates the absence of data and ND that no replicates were available.

Mesocosm		C1	C2	C3	P1	P2	P3	P4	P5	P6	OUT
	Initial $p\text{CO}_2$ (μatm)	378	347	350	494	622	690	477	932	1250	350
	Mean $p\text{CO}_2$ (μatm)	357	356	352	456	486	544	545	719	941	370
Day											
PP-¹⁴C											
3	Mean	0.54	0.53	0.35	0.56	0.47	0.62	0.49	0.44	0.48	0.35
	S.D.	0	0.03	0	0.04	0.03	0.01	0.01	0	0.06	0
5	Mean	0.97	0.78	0.89	0.75	1.07	0.92	1.03	0.92	1.24	0.8
	S.D.	0.01	0.03	0.01	0.08	0.02	0.06	0	0.07	0.01	0.04
7	Mean	0.74	0.67	0.37	0.56	0.77	0.53	0.71	0.7	0.65	0.59
	S.D.	0.08	0.06	0.03	0.01	0.02	0.05	0.16	0.09	0.13	0.03
9	Mean	1.08	1.08	0.91	0.79	1.09	0.92	0.95	0.89	0.96	0.6
	S.D.	0.04	0.17	0.1	0.07	0.05	0.06	0.13	0.02	0.05	0.02
11	Mean	0.54	0.8	0.64	0.74	0.99	0.9	1.06	0.73	1.06	0.43
	S.D.	0.09	0.18	0.04	0.03	0.08	0.2	0.11	0.08	0.11	0.02
DO¹⁴C production											
3	Mean	0.15	0.14	0.16	0.31	0.23	0.13	0.17	0.17	0.38	0.26
	S.D.	0	ND	0	0	0	ND	0	0	0	ND
5	Mean	0.4	0.95	0.28	1.18	0.67	0.47	0.34	0.76	0.37	0.23
	S.D.	0.01	0	0	ND	0.02	0.01	0	0	0	0
7	Mean	0.28	0.19	NA	0.12	0.09	0.16	0.59	0.19	0.21	0.07
	S.D.	0.01	ND	NA	ND	0	0	ND	0	0	ND
9	Mean	0.28	0.16	0.19	0.17	0.15	0.15	0.16	0.18	0.25	0.15
	S.D.	0	0	0	0	0	ND	0	0	0	0
11	Mean	0.26	0.18	0.17	0.29	0.26	0.12	NA	0.21	0.27	0.33
	S.D.	0.01	0	0	0.01	0.01	ND	NA	0	0	ND

Primary production measured with the ¹⁴C labelling technique did not exhibit any change with time and was highly variable from one day to the next (Table II-6). PP-¹⁴C were slightly lower outside than inside the mesocosms and varied from 0.35 ± 0.00 to $0.80 \pm 0.04 \mu\text{mol C L}^{-1} \text{ d}^{-1}$ (Table II-6). During the first part of the experiment (from day 3 to 5), DOC production rates (DO¹⁴Cp) were highly variable both between days and between mesocosms. During the second part of the experiment (from day 7 to the end), this variability decreased and rates were relatively constant with an overall average of $0.21 \pm 0.11 \mu\text{mol C L}^{-1} \text{ d}^{-1}$ (Table II-6). TO¹⁴C production rates (PP-¹⁴C + DO¹⁴Cp) varied from 0.50 ± 0.0 to $2.6 \pm 0.1 \mu\text{mol C L}^{-1} \text{ d}^{-1}$.

PER generally decreased throughout the experiment ($r = -0.58$, $p < 0.05$, $n = 52$) and averaged $25 \pm 12\%$ (from 11 to 61%).

Cumulative PP- ^{14}C , DO ^{14}Cp and TO ^{14}C averaged 7.1 ± 0.8 , 2.6 ± 0.6 and 9.6 ± 0.9 $\mu\text{mol C L}^{-1}$ respectively and did not show any trend with increasing $p\text{CO}_2$ (Figure II-9; PP- ^{14}C : $r = 0.46$, $p > 0.05$, $n = 9$; DO ^{14}Cp : $r = 0.05$, $p > 0.05$, $n = 9$ and TO ^{14}C : $r = 0.38$, $p > 0.05$, $n = 9$, respectively).

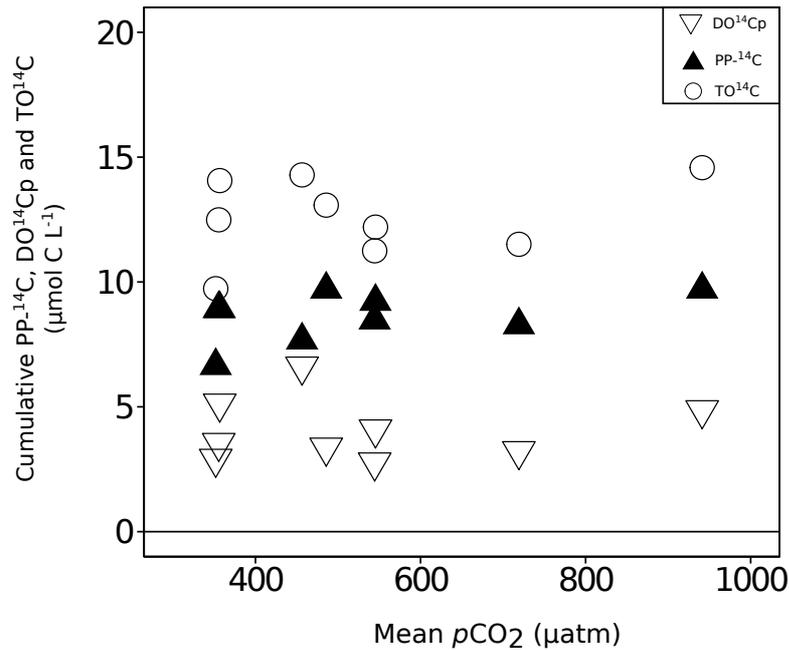


Figure II-9. Cumulative production rates estimated by the ^{14}C method during the experiment in the Bay of Villefranche. PP- ^{14}C : particulate primary production; DO ^{14}Cp : dissolved organic carbon production; TO ^{14}C : total organic carbon production. $p\text{CO}_2$ is the mean value for each mesocosm during the experiment.

Oxygen light-dark and ^{14}C primary production methods were compared without the first day as O $_2$ -LD method provided negative values for NCP that cannot be measured with ^{14}C method. Using data from days 3 to 11, NCP was not significantly correlated with TO ^{14}C rates ($r^2 = 0.06$, $p > 0.05$, $n = 43$) but was correlated with PP- ^{14}C ($r^2 = 0.21$, $p < 0.05$, $n = 45$). PP- ^{14}C was closer to NCP than to GPP-O $_2$ (see comparable cumulative values between NCP and PP- ^{14}C) with GPP-O $_2$ always higher than PP- ^{14}C . Significant correlations were found between GPP-O $_2$ and TO ^{14}C ($r^2 = 0.14$, $p < 0.05$, $n = 43$) and between GPP-O $_2$ and PP- ^{14}C ($r^2 = 0.31$, $p < 0.05$, $n = 45$). PAR at 4 m significantly increased during the experiment from 30 to 190 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Figure II-8; $r = 0.80$, $p < 0.05$, $n = 6$). GPP-O $_2$ and NCP increased significantly with PAR ($r = 0.40$, $p < 0.05$, $n = 54$ and $r = 0.76$, $p < 0.05$, $n = 54$, respectively). For all the results reported, when metabolic rates were normalised to chlorophyll a

concentrations as a best proxy for phytoplankton biomass, the same results were obtained either on the effect of $p\text{CO}_2$ increase or relationship between methods and with PAR.

2.4 Discussion

Characteristics of the study sites

The mesocosms were initially filled with seawater with very low nutrient and chlorophyll concentrations ($\text{NO}_x < 0.10 \mu\text{mol L}^{-1}$; $\text{DIP} < 26 \text{ nmol L}^{-1}$; $\text{chl } a < 0.25 \mu\text{g L}^{-1}$) in the Bay of Calvi to low nutrient and chlorophyll concentrations ($\text{NO}_x < 1.2 \mu\text{mol L}^{-1}$; $\text{DIP} < 20 \text{ nmol P L}^{-1}$; $\text{chl } a < 1.5 \mu\text{g L}^{-1}$) in the Bay of Villefranche. The conditions in BC were typical of the summer stratified period. The initial concentration of nutrients was higher in BV than in BC but nutrients were rapidly consumed and concentrations were relatively low when sampling started. Both experiments were therefore characteristic of low nutrient low chlorophyll areas (LNLC).

Although the availability of nutrient and chlorophyll a concentrations were higher during the spring-winter in BV, GPP rates based on the oxygen light-dark method were similar during the two experiments. This suggests that during the winter period in BV, the community was limited by nutrients as well as light and temperature. The chlorophyll a data obtained at Point B in BV in 2013 revealed that no real bloom occurred that year, although chlorophyll concentrations were maximal in April (Gazeau et al., in prep, a). Although GPP was roughly identical during the two experiments, cumulative NCP was close to 0 in BC, suggesting a metabolic balance. In contrast, cumulative NCP was above 0 in BV, suggesting autotrophy. As a consequence of different trophic states and temperature levels between the two experimental sites and periods, surface waters were a source of CO_2 for the atmosphere in BC (initial $p\text{CO}_2$ of 430 μatm above atmospheric equilibrium; see Gazeau et al., in prep, a) and a sink of CO_2 in BV (initial $p\text{CO}_2$ of 350 μatm below atmospheric equilibrium; see Gazeau et al., in prep, a). The sink status of BV in winter is in agreement with times-series data (De Carlo et al., 2013). The environmental and trophic conditions of the two study sites were oligotrophic summer conditions in BC and pre-bloom mesotrophic conditions in BV.

Metabolic rates measured during both experiments were within the range of previously reported rates in coastal locations of the Mediterranean Sea (Navarro et al., 2004; Gazeau et al., 2005; González et al., 2008; Bonilla-Findji et al., 2010; Ridame et al., 2014) and in open waters (Regaudie-de-Gioux et al., 2009; López-Sandoval et al., 2011). More specifically, the heterotrophic conditions encountered at BC were consistent with the summer heterotrophic conditions reported in the Bay of Palma in 2001 (Navarro et al., 2004; Gazeau et al., 2005).

Furthermore, the values of GPP reported in the present study were below the threshold for metabolic balance (minimum GPP necessary to balance CR) as determined during a Mediterranean Sea transect performed in summer and late-spring of 2006 and 2007 ($4 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$; Regaudie-de-Gioux et al., 2009). Very few data are available using the GPP- ^{18}O method in the Mediterranean Sea, however rates measured in June-July in BC were in the range of those found in BV during the same time period in 2003 by González et al. (2008) and much lower than those determined in March 2012 in BV by Maugendre et al. (accepted, see Chapter IV). In mesocosm experiments conducted during summer 2008 and 2010 in Corsica, primary production rates measured in the control mesocosm using the ^{13}C labelling technique over 24 h ($\sim 0.3 - 0.4 \mu\text{mol C L}^{-1} \text{ d}^{-1}$; Ridame et al., 2014) fall in between our rates of GPP and NCP as this method has been shown to provide rates over 24 h incubations much lower than those estimated with the O_2 -LD technique (Hashimoto et al., 2005).

In BV, GPP- O_2 was lower than the values reported by González et al. (2008) for a similar period in winter-spring. This emphasises the pre-bloom conditions and the likely limitation of metabolic processes by temperature and light. PP- ^{14}C was on average $34 \pm 9\%$ of GPP- O_2 while TO- ^{14}C represented $48 \pm 16\%$ of GPP- O_2 . These percentages are in the range of 40 to 80% reported by Robinson et al. (2006) for the oligotrophic Atlantic Ocean. The release of labelled DOC (DO^{14}Cp) was low but could be measured accurately (S.D. ± 0.002). PER averaged $25 \pm 12\%$ which is close to the value $\sim 20\%$ reported by Marañón et al. (2005) over a wide range of primary production rates and to the value of 23.5% measured in the Almeria-Oran front (Fernández et al., 1993). However, this is slightly lower than values measured in two Mediterranean bays (41%; González et al., 2008) and in the open Mediterranean Sea in June-July 2008 (37%; López-Sandoval et al., 2011).

GPP- O_2 exhibited relatively large changes in BC with a maximum value measured on day 10. It could be related to the high abundance of the cyanobacteria *Synechococcus* spp. and autotrophic picoeukaryotes (Gazeau et al., in prep, this issue). Navarro et al. (2004) have shown that a bloom of *Synechococcus* led to autotrophic conditions in the Bay of Palma in summer 2002. Also, water column stratification and PAR were higher on that day (Gazeau et al., in prep, a). In BV, NCP increased throughout the experiment while GPP- O_2 only increased slightly. The increase in NCP is related to a weaker CR, probably caused by a decrease in particulate organic matter available for the heterotrophs (Celussi et al., in prep).

No correlation was found between GPP measured by the O_2 light-dark and the ^{18}O labelling techniques in BC. This is in agreement with previous results obtained in the Bay of Villefranche by Maugendre et al. (accepted, see Chapter IV), although González et al. (2008)

reported a significant correlation at the same location. However, it must be stressed that the BC experiment was performed during the low productive summer period while González et al. (2008) established their correlation across a much wider range of GPP. In BV, ^{14}C primary production was closer to NCP than GPP as it is expected for 24 h incubations (Marra and Barber, 2004).

Effects of ocean acidification on community metabolism

The effect of ocean acidification was investigated at two sites typical of LNLC with three controls and six CO_2 -enriched levels. Despite different metabolic states (heterotrophy vs autotrophy) and period (summer vs pre-bloom), the same absence of response was observed at the two locations. The analysis of all cumulative rates of particulate and dissolved material indicates a neutral effect of CO_2 enrichment. This suggests that in the short term of the experiments (12 to 20 days) and under nutrient limitation, $p\text{CO}_2$ levels as projected for the next decades may not have significant effects on plankton community metabolism. It is in contrast with the hypothesis of enhanced production with increasing $p\text{CO}_2$ suggested by previous experiments performed at community level under nutrient replete conditions. In fact, with the exception of Yoshimura et al. (2010), all previous experiments have been conducted with high nutrient and/or nutrient addition in cold waters (Table II-7).

The mesocosm experiment performed *in situ* in an Arctic Fjord showed that the plankton community was quite sensitive to an increase in $p\text{CO}_2$, although conclusions diverged depending on the measurement method. Tanaka et al. (2013) found that cumulative NCP was not affected by $p\text{CO}_2$ over the whole experimental period, but was negatively affected after the increase in chlorophyll *a* which followed nutrient addition. Engel et al. (2013) found that primary production measured by ^{14}C uptake significantly increased with increasing $p\text{CO}_2$. Other experiments have been performed using smaller mesocosms in the coastal North Sea (Bergen, Norway) as part of the PeECE project. The three consecutive experiments (2001, 2003 and 2005) showed different effects on primary production. During the first experiment, no effect was found on primary production using the oxygen light-dark method in a bloom dominated by the coccolithophore *Emiliana huxleyi* (Delille et al., 2005). No effects were also found during the second experiment in 2003 (unpublished data; see in Egge et al., 2009). In contrast, an increase of cumulative PP- ^{14}C was measured during a nutrient-induced diatom bloom in a similar experimental set-up and at the same location (Egge et al., 2009; Table II-7). This enhanced production was not detected using the oxygen light-dark method and was attributed to a lack of precision in the measurements or to an absence of effect (Egge et al., 2009). The diverse response of primary production at the same location during the PeECE

project could also be attributed to differences in nutrient concentrations and irradiance which also strongly influence the community composition as well as in phytoplankton community composition. Indeed, as highlighted in a recent study, the initial community composition and ratios between phytoplankton species have more effect than ocean acidification on plankton community functioning (Eggers et al., 2014).

During a microcosm (9 L bottles) experiment performed in the Okhotsk Sea (Yoshimura et al., 2010), nutrient concentrations were below detection limits and chlorophyll *a* concentrations were similar to those observed in the present study ($\sim 0.1 - 0.3 \mu\text{g chl } a \text{ L}^{-1}$; Table 4). While increasing $p\text{CO}_2$ levels had no effect on POC production, DOC accumulation decreased significantly (Yoshimura et al., 2010). The lack of effect on POC production is in agreement with the results reported in the present study but the decrease in DOC accumulation is in contrast with the lack of effect on DO^{14}C_p in the Bay of Villefranche. Such difference might be due to the different timings relative to the bloom. In BC and BV the experiments were performed, respectively, in summer and pre-bloom conditions, while in the Okhotsk Sea the experiment was conducted after the spring-bloom with very low $p\text{CO}_2$ ($\sim 200 \mu\text{atm}$). Furthermore, their range of $p\text{CO}_2$ levels was small, with a maximum $p\text{CO}_2$ of about $590 \mu\text{atm}$) corresponding to one of the lowest $p\text{CO}_2$ used in the present study. The differences in initial plankton community state and assemblages between the experiments might explain that, contrary to the Okhotsk Sea, we did not detect any effect on the dissolved compartment.

Plankton communities in the ocean can be limited or co-limited by macronutrients (Low Nutrient Low Chlorophyll; LNLC) or by micronutrient such as iron (Fe) preventing phytoplankton growth even under high nutrient levels (High Nutrient Low Chlorophyll; HNLC). In contrast to other experiments which focused on the effect of iron addition in combination or not with $p\text{CO}_2$ (Hare et al., 2007; Hopkinson et al., 2010; Sugie et al., 2013), a study was recently performed in iron-limited areas of the Bering sea and of the North Pacific (Yoshimura et al., 2013). While no effect of $p\text{CO}_2$ was found in the North Pacific, in the Bering Sea, quantitative and qualitative changes in the production of particulate and dissolved organic matter were observed with increasing $p\text{CO}_2$. As large cells dominated the community in the Bering Sea while in the North Pacific small eukaryotes were more abundant (Table II-7), Yoshimura et al. (2013) attributed these contrasting responses to differences in plankton community composition (Table II-7) and suggested that oceanic communities dominated by small species are less sensitive to increased $p\text{CO}_2$. Since ocean acidification research begun, it has been hypothesised that phytoplankton species with relatively inefficient carbon

concentration mechanisms (CCMs) will be favoured in future conditions by decreasing the energetic cost for CO₂ acquisition while species with an efficient CCM will be less impacted except if down regulating of CCM activity occur, reducing energetic cost and being advantageous for these species (Rost et al., 2003, 2008; Riebesell et al., 1993). Many studies have tried to evaluate CCMs efficiency of different phytoplankton groups however the diversity of CCMs within and among phytoplankton groups makes relatively difficult to establish a clear rule on which group will be favoured or not (see Rost et al., 2008 and references therein). The plankton communities studied here were dominated by small species (i.e., Cyanobacteria (*Synechococcus* spp.), haptophytes, pelagophytes, cryptophytes and chlorophytes; Gazeau et al., in prep, this issue), which seem to have relatively efficient CCMs under current and future CO₂ condition and were therefore not impacted. However, a theoretical approach led to a different hypothesis (Flynn et al., 2012), suggesting that small phytoplankton species are less adapted to changes in their local pH while larger cells must face larger pH variations at short time scale (day or hours). Our data do not support this hypothesis.

In conclusion, this perturbation experiment was carried out in a typical LNLC area in two sites with different metabolic status (summer and pre-bloom periods). In both experiments, no effect of ocean acidification on community metabolism could be detected. Plankton communities were limited by nutrient availability and an increase in CO₂ concentrations had, not surprisingly, no effect on community metabolic rates. Although the present study was not performed during a phytoplankton bloom, which is very limited in time and biomass in the study area, our results suggest that biological carbon fixation in oligotrophic areas such as the NW Mediterranean Sea will not be enhanced by CO₂ enrichment. If these results hold true for all oligotrophic areas, there would be no negative feedback of the biological pump to atmospheric CO₂ increase. However, short perturbation events stimulating metabolic rates, such as Saharan dust deposition, nutrient fertilization (for example by water column mixing, land run off) could induce a different response to ocean acidification and should be investigated in these areas in the future. Finally, as ocean acidification can act synergistically with other CO₂-related perturbations such as ocean warming, it is of the utmost importance for future studies to consider interactions with other drivers related to climate change.

Table II-7. Effects of ocean acidification as observed during previous experiments under different environmental conditions. The range in nitrogen (NO_x = nitrate + nitrite), phosphate (DIP) and chlorophyll *a* (chl *a*) concentrations as well as temperature (T) and the main phytoplankton groups are presented. LOD: below detection limit and ND: not determined.

Experiment, location and year	T (°C)	NO _x (μmol L ⁻¹)	DIP (μmol L ⁻¹)	chl <i>a</i> (μg L ⁻¹)	Main phytoplankton group	Effect on metabolic rates or main result	Reference
Low nutrient concentrations							
Bay of Calvi, NW Mediterranean (2012)	21.5 to 24.5	< 0.04	< 0.01	0.04 to 0.19	Haptophytes and Cyanobacteria	No effect on community metabolism	this study; pers. comm.
Bay of Villefranche, NW Mediterranean (2013)	13 ± 0.5	< 1.2	< 0.01	0.36 to 1.27	Haptophytes and Cryptophytes	No effect on community metabolism	this study; pers. comm.
Oshtock Sea (2006)	13.5	0.02 to 0.05	0.22 to 0.25	0.2 to 4	<i>Synechococcus</i> spp. and < 5 μm eukaryotes	Respectively, no and less particulate and dissolved organic carbon accumulation	Yoshimura et al. (2010)
Nutrient addition							
PeECE I Bergen (2001)	10 to 13	LOD to 17	LOD to 0.5	1 to 12.5	Temporal shift from <i>Synechococcus</i> spp. to <i>Emiliana hux.</i>	No effect on organic matter production	Delille et al. (2005)
PeECE II Bergen (2003)	8 to 10	LOD to 9	LOD to 0.5	0.2 to 4.2	Temporal shift from <i>E. hux.</i> to diatoms	Small species more affected, no effects on metabolic rates	Engel et al. 2008; (Egge, unpublished data); (Engel, unpublished data)
PeECE III Bergen (2005)	9 to 11.5	LOD to 15	LOD to 0.6	1.5 to 13	Temporal shift from diatoms & <i>E. hux.</i> to flagellates	Increase in primary production (¹⁴ C labelling over 24 h) but no effect on net community production (O ₂)	Egge et al. (2009)

Experiment, location and year	T (°C)	NO _x (μmol L ⁻¹)	DIP (μmol L ⁻¹)	chl <i>a</i> (μg L ⁻¹)	Main phytoplankton group	Effect on metabolic rates or main result	Reference
Svalbard (2010)	2 to 5.5	0.1 to 5.5	0.09 to 0.4	0.22 to 0.31	Haptophytes and Mixotrophes	Respectively no and negative effect on oxygen and ¹³ C measurements methods on net community production, for whole period	Tanaka et al. (2013); Kluijver et al. (2013); Engel et al. (2013)
Iron limited areas							
Bering Sea (2007)	8.4	16	1.5	0.39 to 2.4	Diatoms (65 %)	Increase in particulate organic carbon accumulation	Yoshimura et al. (2013)
North Pacific (2007)	9.2	16	1.4	0.21 to 2.8	Ultraeukaryotes and <i>Synechococcus</i> spp.	No effect on particulate organic carbon accumulation	

Chapter III

Carbon 13 labelling studies and biomarkers analysis on Mediterranean plankton communities

1. Preamble

In the following study, experimental enclosures were enriched with carbon-13 and group-specific incorporation rates were estimated based on the ^{13}C signature of specific molecules: polar lipid fatty acids (PLFA). These PLFA are cell membrane components that allow discriminating between different phytoplankton groups as well as bacteria, i.e. biomarkers. They are synthesized *de novo* during cell growth (Figure III-1) and therefore reflect the production of newly produced material. Furthermore, in contrast to neutral lipids, they have a relatively constant concentration between individuals. Finally, PLFA have short turnover times providing therefore a good indicator of the living biomass.

PLFA terminology is expressed as X:Y ω Z or X:Y (n-Z), X being total number of carbon atoms in the fatty acid, Y is the number of double bonds (or unsaturated bonds) in the chain and Z is the precise position of the first unsaturated bond from the terminal carbon. There are different types of PLFA: saturated FA (SFA; no double bound), mono-unsaturated FA (MUFA) and poly-unsaturated FA (PUFA).

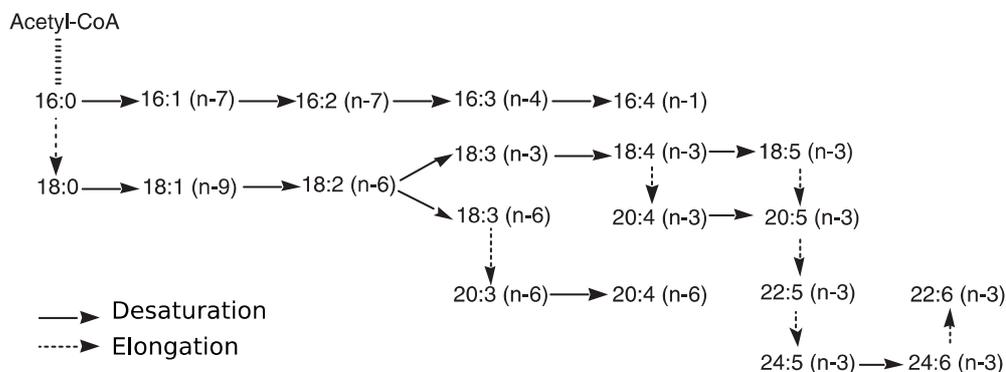


Figure III-1. Major biosynthesis fatty acids pathways in marine algae. Extracted from Dalsgaard et al. (2003), modified after Gurr and Harwood (1991) and Cook (1996).

2. Carbon-13 labelling studies show no effect of ocean acidification on Mediterranean plankton communities

Note: The manuscript as presented here is not the final version but has been reviewed by all co-authors at the time to submit the thesis and will require some modifications before submission during December 2014 in the special issue with other “MedSeA mesocosm experiments” manuscripts. Therefore, if necessary, please refer to the more recent version of the manuscript than the one available at the time of submitting this thesis manuscript.

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Abstract

The effect of ocean acidification on the flow of carbon within a plankton community was investigated in two bays of the Northwestern Mediterranean Sea. In the Bay of Calvi (Corsica, France; summer 2012) and in the Bay of Villefranche (France; winter/spring 2013), nine off-shore mesocosms (~50 m³) were deployed among which 3 served as controls and 6 were enriched with CO₂ to reach *p*CO₂ levels from 450 to 1350 μatm and 350 to 1250 μatm in the Bay of Calvi and the Bay of Villefranche, respectively. The experiments were performed in two different seasons and under contrasting plankton physiological states. At the start of the experiment, all mesocosms were enriched with inorganic ¹³C in order to follow carbon transfer from dissolved inorganic to bulk particulate organic carbon, as well as to phytoplankton (mixotrophs and autotrophs) and to bacteria by means of biomarkers (specific phospholipids fatty acids). Estimated group specific primary production rates and growth rates suggest a relatively higher mixotrophic activity and nutrient remineralisation in the Bay of Calvi (summer) while in the Bay of Villefranche (winter/spring), autotrophic activity was clearly dominating. The increase in *p*CO₂ did not have any effect on total or group specific production rates and on the transfer of recently fixed carbon to heterotrophic bacteria. These experiments were the first conducted in a warm oligotrophic waters and suggest that ocean acidification may not significantly impact plankton carbon flows under nutrient limiting conditions.

Acknowledgments

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2.1 Introduction

Carbon dioxide (CO₂) concentration in the atmosphere are increasing at an unprecedented rate in Earth history due to human activities, warming the lower atmosphere and the ocean. As about 25 % of the emitted CO₂ dissolves in seawater (Le Quéré et al., 2013) forming carbonic acid and releasing protons, the acidity of the ocean is also increasing (i.e. ocean acidification; Gattuso and Hanson 2011). The effect of ocean acidification on plankton community has recently raised important concerns as plankton plays a key role in the global carbon cycle and marine food webs. Primary production, community respiration and organic matter (OM) export to the deep-sea, the so-called biological pump, are the main biological controls of the fluxes of CO₂ from the atmosphere to the ocean. As CO₂ is the main substrate for photosynthesis and as RuBisCO activity is not optimal at the concentrations of CO₂ present in ocean surface waters, primary production could potential benefit from an increase of CO₂ concentrations as a consequence of ocean acidification. However, as RuBisCO enzyme presents low affinity for CO₂ in water, most phytoplankton groups have developed carbon concentration mechanisms (CCMs; Giordano et al., 2005) the efficiency of which is species-dependent (Reinfelder, 2011). It is therefore expected that while some species will be favoured by ocean acidification, others will not, leading to shifts in community structure. Therefore, community compositions as well as primary production of specific phytoplankton groups are important information to elucidate the response carbon fixation and export capacity to ocean acidification. Furthermore, the plankton community comprises autotrophs, heterotrophs and mixotrophs, which are involved in many ecological interactions among which dissolved organic production and consumption that differ between species. Dissolved organic carbon (DOC) production and concentration have been shown to be sensitive to increased CO₂ levels, either positively or negatively (e.g. Yoshimura et al., 2010; Engel et al., 2013) confirming the need to study natural assemblages rather than individual species or strains.

Several experiments have recently been conducted at the community level to assess the effects of ocean acidification on the structure and functioning of plankton communities in different areas of the ocean. So far, these experiments provided variable and sometimes conflicting results, preventing to derive a general concept on the effects of ocean acidification (see Riebesell and Tortell, 2011 for review). For instance, in some studies, ocean acidification has been shown to modify the community structure towards more diatoms (Tortell et al., 2002; 2008) or towards smaller species (Brussaard et al., 2013). In other studies, no changes

were found (Nielsen et al., 2010; 2012). Furthermore, most of these experiments have been conducted in cold nutrient-rich or with experimentally added nutrient concentrations to deliberately induce a bloom. There is therefore a strong lack of data for warm, low nutrient and low productive regions although these areas represent a vast majority of the surface ocean (> 60 %, Longhurst et al., 1995). Also, most of the experiments have been performed during a natural or artificial phytoplankton blooms that only occur during a restricted period of the year and may not reflect the physiological state of plankton community and ecosystem trophic state for most of the year. Therefore the response of plankton communities to ocean acidification under undisturbed conditions, i.e. without nutrient addition and natural assemblages, is poorly known. This work is a contribution towards filling this knowledge gap.

The Mediterranean Sea is oligotrophic for most of the year and locations despite the fact that several bioregions have been identified (D'ortenzio and D'Alcalà, 2009). The decrease in pH in this sea has been estimated to be ~ 0.15 pH units since the industrial revolution (Touratier and Goyet, 2009) and an additional decrease of 0.3 to 0.4 units pH is foreseen for the end of the century (Geri et al., 2014). The effect of ocean acidification on plankton community has been investigated based on mesocosm experiments conducted in two different sites of the Northwestern Mediterranean Sea (Gazeau et al., in prep, a). In this Chapter, we report on the use of inorganic carbon 13 (^{13}C) enrichment to trace the flow of carbon from the inorganic to the organic compartments. In addition to ^{13}C -labelling, analyses of phospholipids fatty acids (PLFA) biomarkers provided taxonomic information. PLFA are cell membrane components produced by phytoplankton and bacteria, that occur in relatively fixed proportion in cells and that allow distinguishing among large groups of organisms (Middelburg, 2014). Moreover, PLFA are rapidly degraded after cell death and therefore largely reflect the activity of living cells (Boschker and Middelburg, 2002). The combination of ^{13}C stable isotope labelling with biomarkers analyses has been used to determine production rates at taxa-specific (Dijkman et al., 2009) and at community level (Van Den Meersche et al., 2004, 2011; De Kluijver et al., 2010; 2013) as it can be performed directly in large mesocosms. This Chapter reports on the first ^{13}C labelling study on plankton communities in the frame of an experiment on ocean acidification effects in the Mediterranean Sea, enabling to test for its limits in low nutrient and relatively unproductive areas.

2.2 Material and Method

2.2.1 Study sites, experimental set-up and sampling

Two mesocosm experiments were carried out in the Bay of Calvi (BC; Corsica, France) in June-July 2012 and in the Bay of Villefranche (BV; France) in February-March 2013. The experimental set-up and mesocosm characteristics are described in Gazeau et al. (in prep, a) and in chapter II of the thesis. In brief, for each experiment, nine mesocosms of ca. 50 m³ (2.5 m in diameter and 12 m maximum depth) were deployed for 20 and 11 days in BC and BV, respectively. Once the bottom of the mesocosms was closed, CO₂ saturated seawater was added in steps over 3 days to obtain a *p*CO₂ gradient from ambient levels to an intended 1,200 µatm, with three control mesocosms (C1, C2 and C3) and six mesocosms with increasing *p*CO₂ (P1 to P6). 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 ‰.

Every morning, depth-integrated samplings (0 – 10 m) were performed using 5 L Hydro-Bios integrated water samplers and sampled seawater was used for various analyses such as particulate organic matter (Gazeau et al., in prep, c), nutrient (Louis et al., in prep) and pigment concentrations (Gazeau et al., in prep, b). Every other day, samples were taken for microbial diversity analyses, performed using flow cytometry techniques (Celussi et al., in prep). Daily samples for δ¹³C-DIC, δ¹³C-particulate organic carbon (δ¹³C-POC) and δ¹³C-phospholipid (polar) fatty acid (δ¹³C-PLFA) analyses were taken at the beginning (day 0 to 15 in BC and day 0 to 4 in BV) and every second day toward the end of the experiments. The sediments traps were emptied every day in BC or every other day in BV and samples were immediately preserved with pH buffered formol. In BC, the final zooplankton net haul (200 µm mesh size) was performed in each mesocosm at the end of the experiment. Unfortunately, a storm caused an unintended opening of the mesocosms on day 13 (Gazeau et al., in prep, a for details; and in chapter II) no zooplankton net haul could be done in BV.

Samples for δ¹³C-POC were immediately filtered on pre-weighted and pre-combusted 25 mm GF/F using 0.5 to 1 L of collected seawater. Filters were dried at 60 °C and stored in a dry place pending analysis. For δ¹³C-DIC analyses, 20 mL of seawater was gently transferred to glass vial avoiding bubbles and vials were sealed after being poisoned with 10 µL saturated HgCl₂ and stored upside-down at room temperature in the dark pending analysis. The δ¹³C-

PLFA samples were gently filtered through 47 mm pre-combusted GF/F filters using a known volume of seawater of ca. 4 L of seawater and were stored at -80°C . Zooplankton samples of the final net haul were transferred to filtered seawater for half an hour to empty their guts. One to ten individuals of the two species *Paracalanus* spp. and *Oncaea* spp., that were found in nearly all mesocosms, were transferred to pre-combusted tin cups and were stored at -80°C for later organic $\delta^{13}\text{C}$ content analyses. For sediment trap samples, swimmers larger than 1 mm were removed and the remaining samples were rinsed, centrifuged and freeze-dried. In BC, as a consequence of low amounts of material especially at the end of the experiment, daily samples were pooled as follows: d5-7, d8-10, d11-14 and d15-19. Total particulate matter was weighed for flux determination and subsamples were used for POC and $\delta^{13}\text{C}$ -POC measurements. At the time of presenting the manuscript for this thesis, POC concentration and ^{13}C -POC measurements in sediment traps for the experiment in BV are still being processed.

2.2.2 Laboratory analysis

All sample preparations and measurements for $\delta^{13}\text{C}$ analyses were performed at the Netherlands Institute of Sea Research (NIOZ-Yerseke; The Netherlands). POC samples were analyzed for organic carbon content and isotope ratios on an elemental analyser (Thermo Electron Flash EA 1112) coupled to a Delta V isotope ratio mass spectrometer (IRMS). For $\delta^{13}\text{C}$ -DIC analyses, a helium headspace (3 mL) was created in the vials and samples were acidified with 2 μL of phosphoric acid (H_3PO_4 , 99%) to transfer all DIC to gaseous CO_2 . After equilibration, the CO_2 concentration in the headspace and its isotopic composition were measured on an EA-IRMS. PLFA were extracted using a modified Bligh and Dyer method (Middelburg et al., 2000). In brief, after total lipids extraction in a methanol:chloroform mix, lipids were separated into different polarity classes on a column separation using previously heat activated silica. After elution with chloroform and acetone, the methanol fraction was collected and PLFA were derivatized to fatty acid methyl esters (FAME). The standards 12:0 and 19:0 were used as internal standards. Concentrations and $\delta^{13}\text{C}$ of individual PLFA were measured using gas chromatography-combustion isotope ratio mass spectrometry (GC-c-IRMS; Middelburg et al., 2000; De Kluijver et al., 2010; 2013). In BC, due to very low concentrations, the daily PLFA samples were pooled by two days after the extraction step.

2.2.3 Data analysis

Carbon isotope data are expressed in the delta notation (δ) relative to Vienna Pee Dee Belemnite (VPDB) standard and are presented as specific enrichment ($\Delta\delta^{13}\text{C}$) and ^{13}C

incorporation (Middelburg, 2014). The specific enrichment Δ was calculated as $\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{background}}$ with $\delta^{13}\text{C}_{\text{background}}$ being the isotope ratio under natural conditions (before ^{13}C addition). The carbon isotope ratio was calculated as $R_{\text{sample}} = (\delta^{13}\text{C}_{\text{sample}}/1000+1) \times R_{\text{VPDB}}$, with $R_{\text{VPDB}} = 0.011237$. The ^{13}C fraction was calculated as: $^{13}\text{F} = ^{13}\text{C}/(^{13}\text{C}+^{12}\text{C}) = R/(R+1)$. The excess ^{13}C was obtained as $\Delta^{13}\text{F} = ^{13}\text{F}_{\text{sample}} - ^{13}\text{F}_{\text{background}}$. Incorporation were then calculated as $^{13}\text{C}\text{-concentration} = \Delta^{13}\text{F} \times \text{C}$ ($\mu\text{mol L}^{-1}$; De Kluijver et al., 2010) with C being POC or PLFA concentrations in $\mu\text{mol L}^{-1}$. In order to directly compare values between mesocosms, data were corrected for the different initial $\delta^{13}\text{C}\text{-DIC}$ using a correction factor calculated as the ratio between $\delta^{13}\text{C}\text{-DIC}$ in each mesocosm to the average $\delta^{13}\text{C}\text{-DIC}$ in all mesocosms at d0. This ratio varied from 0.92 to 1.21 in BC and from 0.72 to 1.22 in BV. $\delta^{13}\text{C}\text{-DIC}$ data were corrected for air-sea gas exchanges using the method described in De Kluijver et al. (2013).

The PLFAs ai15:0 and i15:0 were used as specific biomarkers for gram-positive and -negative bacteria although the more abundant 18:1 ω 7c is sometimes used for gram-negative bacteria but this PLFA can also be found in some phytoplankton species. For phytoplankton, different PLFAs were detected depending on the site and higher PLFA concentrations and more diversity were detected in BV than in BC. Based on the dynamics of ^{13}C enrichment, two phytoplankton groups were considered: one with fast and one with delayed incorporation. In BC, PLFA that showed delayed incorporation were 16:2 ω 4, 20:4 ω 6, 20:5 ω 3 and 22:6 ω 3 and these are characteristic for diatoms and (mixotrophic) dinoflagellates. Their concentration-weighted $\delta^{13}\text{C}$ and sum of concentrations were used to describe a general group considered as mixotrophs (Dalsgaard et al., 2003; Dijkman et al., 2009; De Kluijver et al., 2013). PLFAs that showed quick incorporation were 18:4 ω 3 and 18:3 ω 3 and their weighted isotope value was used for autotrophic phytoplankton with chlorophytes and haptophytes. Similarly, in BV, a slowly incorporating group containing 16:4 ω 3, 16:4 ω 1; 20:5 ω 3 and 22:6 ω 3 was defined as mixotrophs (i.e. diatoms, dinoflagellates and prae-sinophytes). The fast incorporating group (18:3 ω 3, 18:4 ω 3, 18:5 ω 3(12-15) and 18:5 ω 3(12-16)) was used to characterize autotrophs, with chlorophytes, haptophytes, cryptophytes and autotrophic dinoflagellates. The sum of characteristic PLFA concentrations were converted to total carbon concentration using conversion factors ($\mu\text{g C PLFA} / \mu\text{g C cell}$) of 0.01, 0.06 and 0.05 for bacteria, autotrophs (high incorporation rates) and mixotrophs (low incorporation rates), respectively (Van Den Meersche et al., 2004; Dijkman et al., 2009; De Kluijver et al., 2013). Bacterial biomass was also converted to cell abundance using a carbon content of 20 fg cell^{-1} (Lee and Fuhrman, 1987).

Primary production rates were calculated based on ^{13}C incorporation in POC as well as in PLFA characteristic of each phytoplankton group (autotrophs and mixotrophs) and bacteria, using the equation:

$$PP = [(\Delta \text{}^{13}\text{F}_{\text{biomass}} * C_{\text{biomass}}) / \Delta t - (\text{}^{13}\text{F}_{\text{mean;biomass}} * \Delta C_{\text{biomass}}) / \Delta t] / [\text{}^{13}\text{F}_{\text{mean;DIC}} - \text{}^{13}\text{F}_{\text{mean;biomass}}]$$

in $\mu\text{mol C L}^{-1} \text{d}^{-1}$ with, $\text{}^{13}\text{F}_{\text{biomass}}$ the ^{13}C fraction in the considered biomass (PLFA autotrophs, mixotroph, bacteria or POC), C_{biomass} the concentration of the considered biomass in $\mu\text{mol C L}^{-1}$; Δt is the time interval in days (d^{-1}), $\text{}^{13}\text{F}_{\text{mean;biomass}}$ is the average ^{13}C fraction in the considered biomass (PLFA or POC) for the time interval and $\text{}^{13}\text{F}_{\text{mean;DIC}}$ is the average ^{13}C fraction in DIC for the considered time interval.

2.2.4 Model

Having isotope enrichment data ($\Delta\delta^{13}\text{C}$) at multiple time steps allows using simple sink-source isotope ratio model based on that of Hamilton et al. (2004) in which the isotopic composition of a consumer is altered by the uptake of the source compartments minus any losses. This model is based on the assumption that biomass of consumers is constant with time and allows estimating the turnover rate of the phytoplankton and bacterial groups (r). Here we apply a phytoplankton-bacteria-detritus model among which two phytoplankton types are considered (Phyto1 and Phyto2). For this system, the set of equations reads:

$$d \Delta \delta^{13}\text{C}_{\text{Phyto1}} / d t = r_{P1} (\Delta \delta^{13}\text{C}_{\text{DIC}} - \Delta \delta^{13}\text{C}_{\text{Phyto1}})$$

$$d \Delta \delta^{13}\text{C}_{\text{Phyto2}} / d t = r_{P2} (\Delta \delta^{13}\text{C}_{\text{DIC}} - \Delta \delta^{13}\text{C}_{\text{Phyto2}})$$

$$d \Delta \delta^{13}\text{C}_{\text{bact}} / d t = r_{bac} (\Delta \delta^{13}\text{C}_{\text{Phyto1}} - \Delta \delta^{13}\text{C}_{\text{bact}})$$

$$d \Delta \delta^{13}\text{C}_{\text{det}} / d t = r_{\text{Phyto1}} (\Delta \delta^{13}\text{C}_{\text{Phyto1}} - \Delta \delta^{13}\text{C}_{\text{det}}) + r_{\text{Phyto2}} (\Delta \delta^{13}\text{C}_{\text{Phyto2}} - \Delta \delta^{13}\text{C}_{\text{det}}) + r_{bac} (\Delta \delta^{13}\text{C}_{\text{bact}} - \Delta \delta^{13}\text{C}_{\text{det}})$$

This model was implemented in R software (r core team 2013) using packages FME and deSolve (Soetaert and Petzoldt, 2010; Soetaert et al., 2010) and was applied to the global experimental periods. Some more details on the model can be obtained in Van Oevelen et al. (2006) and De Kluijver et al. (2010). This simple modelling approach allows derivation of model parameters (with uncertainty), which then can be regressed against CO_2 level to test for an ocean acidification effect. By the end of the experiment stable isotope patterns approached steady state and the ratio of the enrichment in consumers ($\Delta\delta^{13}\text{C}_{\text{cons}}$) to the enrichment of the substrate ($\Delta\delta^{13}\text{C}_{\text{subst}}$) can then be used to quantify the dependency of consumers on the resource.

2.2.5 Statistics

Results on isotope ratio, isotope concentrations and biomasses are reported as average \pm SD of the nine mesocosms. Cumulative production was calculated as the sum of production rates calculated from the equation for the available experimental period. Linear interpolation was used for the days when no production rates are available. The $p\text{CO}_2$ used for the representation of cumulative productions were the average $p\text{CO}_2$ over the considered experimental period for each mesocosm. Linear regressions of cumulative rates against $p\text{CO}_2$ were used to test for ocean acidification effects. Model-II linear regressions were used to compare PLFA and chlorophyll *a* concentration. All regressions were performed using the R software (version 3.1; www.r-project.org) and were considered significant at a probability $\alpha = 0.05$.

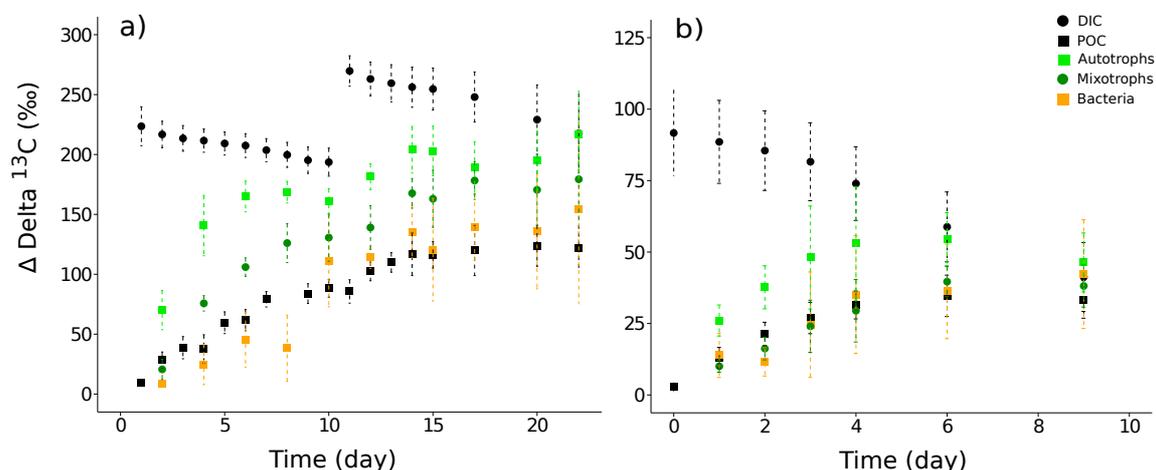


Figure III-2. Average $\Delta\delta^{13}\text{C}$ in the nine mesocosms deployed in a) the bay of Calvi in summer 2012 and b) the bay of Villefranche in winter/spring 2013 reported as mean \pm SD for dissolved inorganic carbon (DIC; black points); particulate organic carbon in the water column (POC; black square), autotroph phytoplankton (light green square), mixotrophs (dark green points) and bacteria (orange square).

2.3 Results

2.3.1 Bay of Calvi

Labelling results: DIC and POC

The addition of $\text{NaH}^{13}\text{CO}_3$ led to an increase of $\Delta\delta^{13}\text{C}$ -DIC in all mesocosms to an average 224 ± 16 ‰ that steadily decreased to a minimum of 194 ± 12 ‰ at d10 before the second addition was performed. This latter further increased $\Delta\delta^{13}\text{C}$ -DIC to 270 ± 13 ‰

(Figure III-2 a). The ^{13}C -DIC concentration varied during the whole experimental period from 7.3 to 4.2 $\mu\text{mol } ^{13}\text{C L}^{-1}$ and accounted for 0.19 to 0.30 % of total DIC concentration and followed the same pattern (Figure III-3a) as described for $\Delta\delta^{13}\text{C}$ -DIC. The decrease in ^{13}C -DIC concentrations occurred in all mesocosms independent of $p\text{CO}_2$ level. Losses through air-sea exchange were negligible ($< 0.7\%$ ^{13}C -DIC; data not shown).

Incorporation into POC was rapid and a first plateau starting at d9 was reached with an average $\Delta\delta^{13}\text{C}$ -POC of $86 \pm 8\%$. The second addition of $\text{NaH}^{13}\text{CO}_3$ on d11 led to a further increase in $\Delta\delta^{13}\text{C}$ -POC until d15 when a second plateau was reached ($122 \pm 18\%$; Figure III-2a). The ^{13}C -POC concentration (Figure III-3b) varied, following the same pattern as $\Delta\delta^{13}\text{C}$ -POC, from 3.6 to $58.2 \cdot 10^{-4} \mu\text{mol } ^{13}\text{C L}^{-1}$. Average ratio of $\Delta\delta^{13}\text{C}$ -POC / $\Delta\delta^{13}\text{C}$ -DIC reached a maximum of ca. 0.54 at the end of the experiment (Table III-1), but remained below 1 implying that not all the particulate material had been labelled during the experimental period.

Phytoplankton and bacteria dynamic: labelling and biomass

The averaged $\Delta\delta^{13}\text{C}$ -mixotroph steadily increased until d12 to $139 \pm 18\%$ and the second $\text{NaH}^{13}\text{CO}_3$ addition on d11 allowed an increase to $179 \pm 36\%$ (Figure III-2a). The autotrophs incorporated label much faster and on d6 a first saturation plateau was reached at $165 \pm 11\%$. After the second $\text{NaH}^{13}\text{CO}_3$ addition, $\Delta\delta^{13}\text{C}$ -autotroph increased again until the end of the experiment to $217 \pm 36\%$ (Figure III-2a). The $\Delta\delta^{13}\text{C}$ -bacteria steadily increased to reach a final average maximum of $155 \pm 24\%$ (Figure III-2a). The ^{13}C content of autotrophs, mixotrophs and bacteria increased (Figure III-4a, b, c) during the experiment, irrespective of the $p\text{CO}_2$ level. The ratio of $\Delta\delta^{13}\text{C}$ -all phytoplankton_(mixotrophs+autotrophs) / $\Delta\delta^{13}\text{C}$ -DIC reached an averaged maximum of 0.78 ± 0.04 on d20 while the averaged ratio $\Delta\delta^{13}\text{C}$ -bacteria / $\Delta\delta^{13}\text{C}$ -DIC was 0.59 ± 0.21 (Table III-1). $\Delta\delta^{13}\text{C}$ -bacteria / $\Delta\delta^{13}\text{C}$ -all phytoplankton averaged 0.80 ± 0.15 at the end of the experiment. The model fit to the data (Figure III-5) and did provide an average growth rates value for all the mesocosm of 0.50, 0.20 and 0.10 d^{-1} for autotrophs, mixotrophs and bacteria respectively and were independent of $p\text{CO}_2$ levels. The bacteria isotope ratio ($\Delta\delta^{13}\text{C}$) was below isotope ratio of autotrophs and mixotroph. The models fit similarly if bacteria are parameterised to grow on auto- or mixo-trophs.

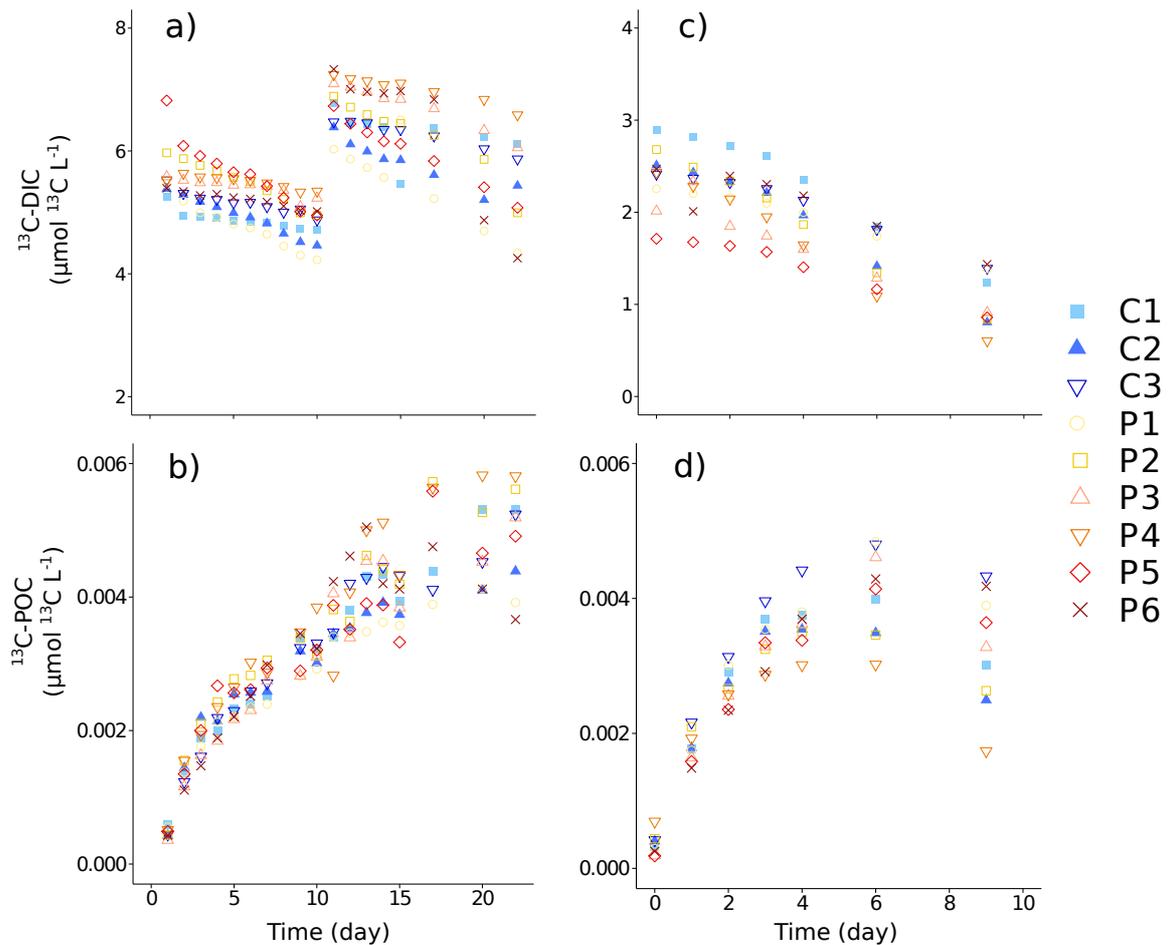


Figure III-3. ^{13}C -biomass ($\mu\text{mol } ^{13}\text{C L}^{-1}$) in the nine mesocosms (C1 to P6) deployed in the Bay of Calvi in summer 2012 (left) and in the Bay of Villefranche in winter/spring 2013 (right) for dissolved inorganic carbon (DIC; upper panels) and particulate organic carbon (POC; lower panels).

The biomass of mixotrophs increased over the experimental period from 0.025 to 0.15 $\mu\text{mol C L}^{-1}$ while the biomass of autotrophs was lower and varied from ~ 0.01 to 0.04 $\mu\text{mol C L}^{-1}$ (Figure III-6a). The dynamics of chlorophyll *a* (Gazeau et al., in prep, b) and total phytoplankton biomass based on PLFA were similar and both variables were significantly correlated between ($n = 106$, $r^2 = 0.14$, $p < 0.01$). Bacterial biomass calculated based on PLFA varied from 0.025 to 0.10 $\mu\text{mol C L}^{-1}$ (Figure III-6a) and converted to cell abundance was higher than measured by flow cytometer (FC) by one order of magnitude and PLFA and FC presented a similar dynamics (data not shown). The biomass based on PLFA for phytoplankton, mixotrophs and bacteria varied independently of $p\text{CO}_2$ levels.

Primary production based POC and PLFA

Based on POC labelling, net production rates (NCP-¹³C) varied from 0.00 to 1.02 $\mu\text{mol C L}^{-1} \text{d}^{-1}$ with large variations between mesocosms and sampling days (data not shown). Cumulative productions ranged from 2.62 to 3.24 $\mu\text{mol C L}^{-1}$ (Figure III-7) with no significant trend with increasing $p\text{CO}_2$ ($n = 9$; $r = 0.05$; $p > 0.05$). Mixotroph production rates (delayed incorporation group) varied between 0.00 and 0.03 $\mu\text{mol C L}^{-1} \text{d}^{-1}$. The lowest cumulative productions were measured in P5 and P6 (0.02 $\mu\text{mol C L}^{-1}$) and the highest was measured in P2 (0.12 $\mu\text{mol C L}^{-1}$; Table III-2). These cumulative productions rates did not show any trend with increasing $p\text{CO}_2$ levels ($n = 9$; $r = 0.14$; $p > 0.05$). As autotrophic biomass was much lower, it was very difficult to obtain precise estimates of autotroph primary production rates, especially in C2 that was not taken into consideration. Calculated autotroph production rates varied from 0.000 to 0.005 $\mu\text{mol C L}^{-1} \text{d}^{-1}$. Cumulative production ranged from 0.00 to 0.03 $\mu\text{mol C L}^{-1}$ with no significant CO_2 effect (Table III-2; $n = 9$; $r = 0.09$; $p > 0.05$). No dynamics was observed for NCP-¹³C but group-specific phytoplankton production rates based on PLFA showed an identical pattern for both groups: i.e. from d2 to d8, production rates tended to decrease and then increased until d12. After that day, the variability among mesocosms was too important and hid any general dynamics. Bacterial production rates were close to 0 over the experiment with cumulative values ranging from < 0.01 to 0.04 $\mu\text{mol C L}^{-1}$ and no significant effect of increasing $p\text{CO}_2$ (Table III-2; $n = 9$; $r = 0.04$ $p > 0.05$).

Table III-1. Final ratio for the ¹³C enrichment of different particulate organic compartments: bulk particulate organic carbon (POC), all phytoplankton (phyto = autotrophs + mixotrophs) and bacteria, relative to final ¹³C enrichment of dissolved inorganic carbon (DIC) or phytoplankton.

	Bay of Calvi	Bay of Villefranche
$\Delta\delta^{13}\text{C-POC} / \Delta\delta^{13}\text{C-DIC}$	0.54 ± 0.04	0.89 ± 0.10
$\Delta\delta^{13}\text{C-phyto} / \Delta\delta^{13}\text{C-DIC}$	0.78 ± 0.04	1.04 ± 0.12
$\Delta\delta^{13}\text{C-bact} / \Delta\delta^{13}\text{C-DIC}$	0.59 ± 0.21	0.80 ± 0.13
$\Delta\delta^{13}\text{C-bact} / \Delta\delta^{13}\text{C-phyto}$	0.80 ± 0.15	ND

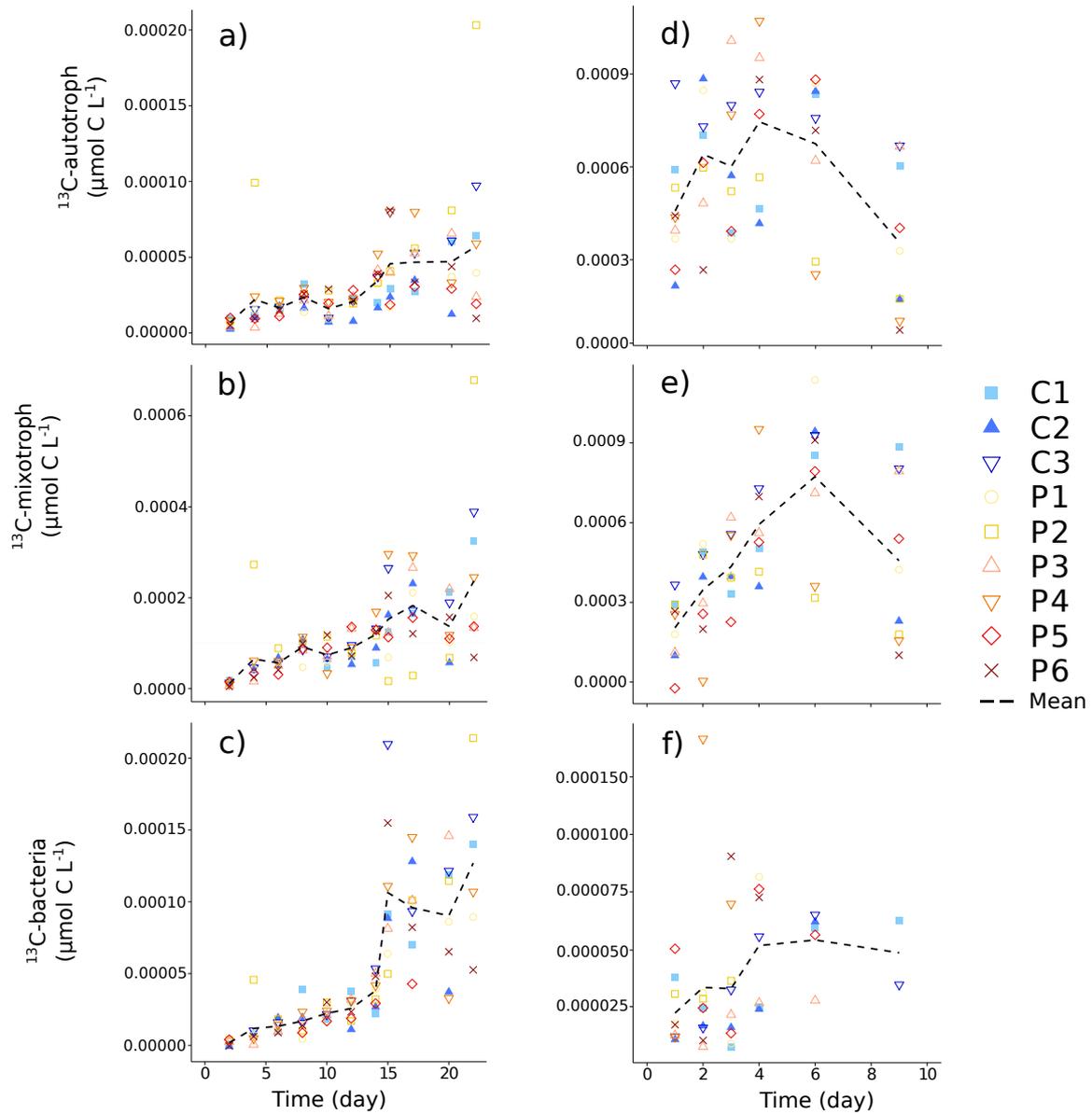


Figure III-4. ^{13}C -biomass ($\mu\text{mol } ^{13}\text{C L}^{-1}$) in the nine mesocosms (C1 to P6) and average (dashed line) deployed in the Bay of Calvi in summer 2012 (left) and in the Bay of Villefranche in winter/spring 2013 (right) for autotroph (upper panels), mixotrophs (middle panels) and bacteria (lower panels).

Zooplankton and sediment traps

Specimens of the copepods *Paracalanus* spp. were present in samples from all mesocosms except P1 and P2 while *Oncaea* spp. was not found in samples from mesocosm P3. *Paracalanus* has shown a higher specific enrichment ($\Delta\delta^{13}\text{C}$; average 108 ± 10 ‰) than *Oncaea* (average 60 ± 10 ‰). Both species were less labelled in P6 (the highest $p\text{CO}_2$ treatment) but there was no significant effect of $p\text{CO}_2$ on zooplankton ^{13}C enrichment (Figure III-8; *Paracalanus*: $n = 7$, $r = -0.73$, $p > 0.05$; *Oncaea*: $n = 8$, $r = -0.31$, $p > 0.05$). Export to sediment traps was fast as after 2 days an increased in ^{13}C was measured (Figure III-9). The cumulated ^{13}C -POC in sediment traps increased steadily independent of $p\text{CO}_2$ level (linear regression on daily cumulated labelled material: $n = 9$, $r = 0.33$, $p > 0.05$). Despite no significant differences between mesocosm, C2 and P1 presented lower values than the other mesocosms.

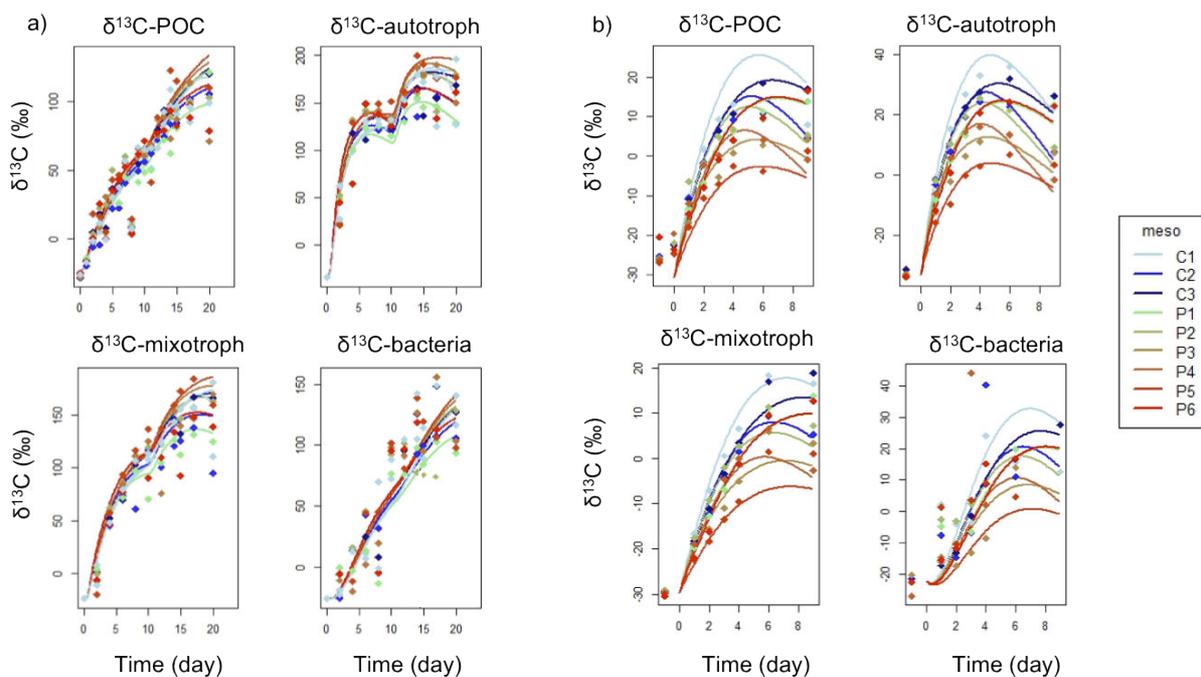


Figure III-5. The model output (solid lines) fitted to the data (points) for all mesocosms deployed a) in the Bay of Calvi in summer 2012 and b) in the Bay of Villefranche in winter/spring 2013. $\delta^{13}\text{C}$ of particulate organic carbon (POC), autotrophs, mixotrophs and bacteria based on polar lipids fatty acid (PLFA) ^{13}C incorporation.

2.3.2 Bay of Villefranche

Labelling results: DIC and POC

The addition of $\text{NaH}^{13}\text{CO}_3$ led to an increase in $\Delta\delta^{13}\text{C-DIC}$ to 92 ± 15 ‰ that steadily decreased to a minimum of 34 ± 11 ‰ until d11 (Figure III-2b). The $^{13}\text{C-DIC}$ concentration varied during the whole experimental period between 0.5 and $2.9 \mu\text{mol } ^{13}\text{C L}^{-1}$, accounting for 0.02 to 0.12 % of total DIC and followed the same pattern (Figure III-3c) as described for $\Delta\delta^{13}\text{C-DIC}$. Losses by air-sea exchange calculated during the experiment were important and were depended on the considered mesocosm. Control mesocosms presented similar negative air-sea fluxes while perturbed mesocosms (P1 to P6) presented positive fluxes with a ^{13}C outgasing up to 3 % of $^{13}\text{C-DIC}$ in the most acidified mesocosms (P5 and P6). This degassing can explain part of the rapid decrease in $^{13}\text{C-DIC}$ observed during the experiment.

Incorporation into POC was rapid and on d6 a plateau was reached with $\Delta\delta^{13}\text{C-POC}$ (average 35 ± 7 ‰; Figure III-2b). $^{13}\text{C-POC}$ concentrations varied, following the same pattern as $\Delta\delta^{13}\text{C-POC}$, from 3.6 to $58.2 \cdot 10^{-4} \mu\text{mol } ^{13}\text{C L}^{-1}$ (Figure III-3d). The ratio of $\Delta\delta^{13}\text{C-POC} / \Delta\delta^{13}\text{C-DIC}$ reached a maximum of ca. 0.90 (Table III-1) at the end of the experiment when nearly all the particulate material had been labelled.

Phytoplankton and bacteria dynamic: biomass and labelling

The $\Delta\delta^{13}\text{C-mixotrophs}$ steadily increased until d6 to 39 ± 5 ‰ while $\Delta\delta^{13}\text{C-autotrophs}$ reached 53 ± 8 ‰. $\Delta\delta^{13}\text{C-bacteria}$ was similar to $\Delta\delta^{13}\text{C-mixotroph}$ with $\Delta\delta^{13}\text{C}$ of 36 ± 5 ‰ on d6 (Figure III-2b). After that day, $\Delta\delta^{13}\text{C-DIC}$, POC and PLFA were at isotopic equilibrium and no other $\text{NaH}^{13}\text{CO}_3$ addition could be done to stimulate further ^{13}C incorporation into particulate matter (Figure III-2b) due to the storm (see 2.2 Material and Method). The $^{13}\text{C-biomasses}$ have shown more variability between mesocosms than during the experiment in the Bay of Calvi and varied independently of $p\text{CO}_2$ level (Figure III-4 d, e, f). Bacteria were very difficult to detect with PLFA during this experiment (Figure III-4f). Ratio of $\Delta\delta^{13}\text{C-all phytoplankton} / \Delta\delta^{13}\text{C-DIC}$ reached an averaged maximum of 1.04 ± 0.12 meaning that all ^{13}C was incorporated into particulate phytoplankton biomass (Table III-1). A final $\Delta\delta^{13}\text{C-bacteria} / \Delta\delta^{13}\text{C-DIC}$ ratio could not be calculated as bacterial PLFA and isotope analyses failed for the samples toward the end of the experiment due to problems during PLFA extraction. The model implemented for the first 9 days (Figure III-5b) has provided growth rates of 0.40 d^{-1} for autotroph, 0.12 d^{-1} for mixotrophs and 0.50 d^{-1} for bacteria. The model implies that bacteria derive their DOC from autotrophs as bacterial isotope ratio was similar or higher than isotope ratio of mixotrophs.

The biomass estimated with PLFA for the two phytoplankton groups were higher than in Bay of Calvi (10 to 20 fold) and tended to decrease over the course of the experiment (9 days; Figure III-6b) with large variability between mesocosms. Furthermore, autotrophs and mixotrophs phytoplankton showed similar concentrations. The biomass from chlorophyll *a* (data not shown; Gazeau et al., in prep, b) and PLFA were not significantly correlated ($n = 60$, $r^2 = 0.06$, $p > 0.05$). Bacterial biomass based on PLFA was about the same as in Bay of Calvi but were difficult to measure during this experiment and large differences between mesocosms were observed (Figure III-6b) masking any general temporal dynamics. The bacterial PLFA concentration converted to cell number (total average $6.6 \cdot 10^6$ cells mL^{-1}) were one order of magnitude higher than bacterial abundance determined by flow cytometry (total average $8.3 \cdot 10^5$ cells mL^{-1}) showing an increase in abundance from d4 to d9. The biomass based on PLFA for autotrophs, mixotrophs and bacteria varied independently of $p\text{CO}_2$ levels.

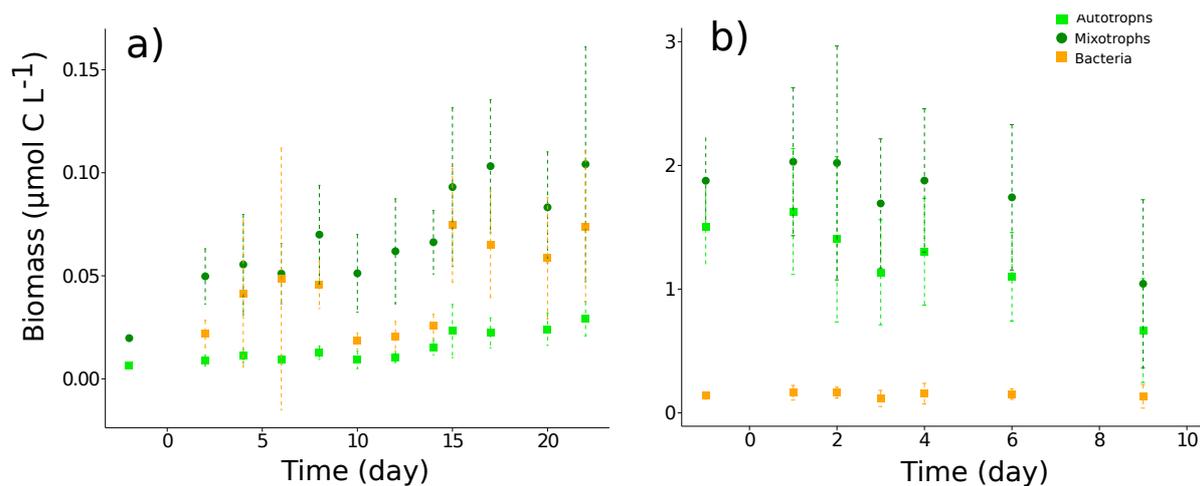


Figure III-6. Average biomass concentration in all nine mesocosms deployed in the Bay of Calvi (summer 2012; a) and in the Bay of Villefranche (winter/spring 2013; b) for bacteria (orange full squares), mixotrophs (green full circles) and autotrophs (green empty circles).

Primary production based POC and PLFA

Net community production based on ^{13}C -POC incorporation (NCP- ^{13}C) was decreasing over the experiment. At d0, NCP- ^{13}C averaged $1.04 \pm 0.22 \mu\text{mol C L}^{-1} \text{d}^{-1}$ (slightly higher than measured by ^{14}C -PP; $0.85 \pm 0.18 \mu\text{mol C L}^{-1} \text{d}^{-1}$; see Chapter 2) and on d6 reached a minimal value of $-0.09 \pm 0.41 \mu\text{mol C L}^{-1} \text{d}^{-1}$. As ^{13}C -POC equilibrated with ^{13}C -DIC already on d6, it was not possible to calculate production rates after that day. Cumulative production from day 0 to 6 varied from 2.9 to 6.3 $\mu\text{mol C L}^{-1}$ in P4 and C3 respectively and

were not correlated to increased $p\text{CO}_2$ (Figure III-7; $n = 9$, $r = -0.09$, $p > 0.05$).

Mixotroph production rates were constant during the four first days of the experiment and averaged $0.12 \pm 0.03 \mu\text{mol C L}^{-1} \text{d}^{-1}$ during the whole experiment. Cumulative rates ranged from 0.50 (in P1 and P6) to 0.91 (in C3) $\mu\text{mol C L}^{-1}$ and were not significantly correlated with increasing $p\text{CO}_2$ levels (Table III-2; $n = 9$, $r = -0.06$, $p > 0.05$). Contrary to mixotrophs, autotrophs showed a general decrease in production rates over the course of the experiment (from $0.28 \pm 0.05 \mu\text{mol C L}^{-1} \text{d}^{-1}$ on d0 to $0.02 \pm 0.04 \mu\text{mol C L}^{-1} \text{d}^{-1}$ on d4). Cumulative production did not show any trend with increasing $p\text{CO}_2$ levels ($n = 9$, $r = -0.44$, $p > 0.05$) and ranged from 0.66 to 1.24 $\mu\text{mol L}^{-1}$ in P6 and C3 respectively (Table III-2).

Zooplankton and sediment traps

As mentioned in the Material and Method section, no samples were available for zooplankton. At the time of submitting this thesis, sediment traps samples from the experiment in BV have been prepared and analyses for ^{13}C -POC and POC will be performed in before the end of 2014 at the Laboratoire d'Océanographie de Villefranche. These data will be incorporated in the present manuscript for submission in December 2014.

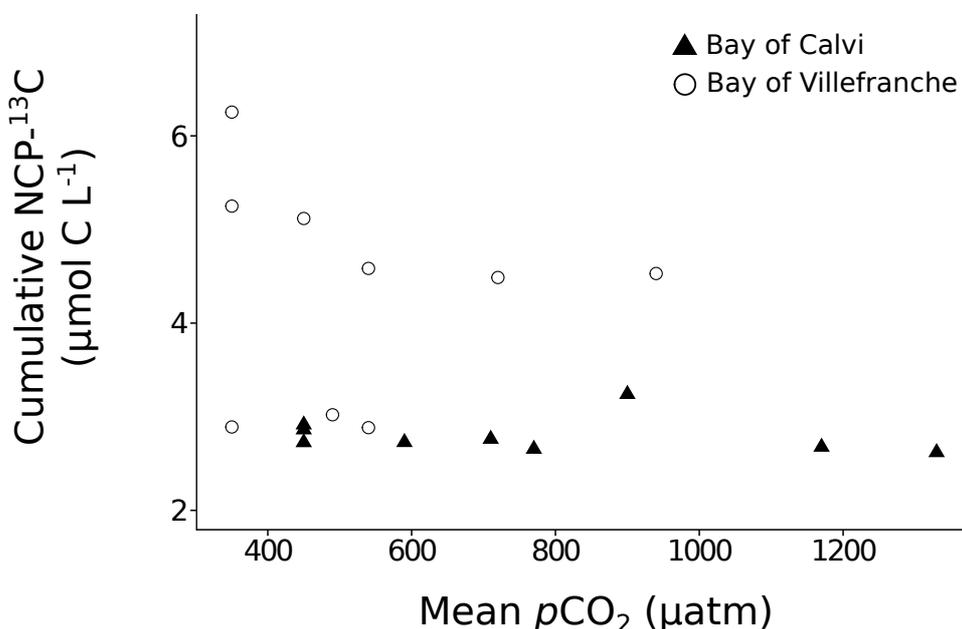


Figure III-7. Cumulative net community production rates based on production rates) based on ^{13}C -POC incorporation (NCP- ^{13}C ; $\mu\text{mol C L}^{-1}$) as a function of averaged $p\text{CO}_2$ levels during the experimental periods considered, in the Bay of Calvi (full triangles) and Villefranche (empty circles).

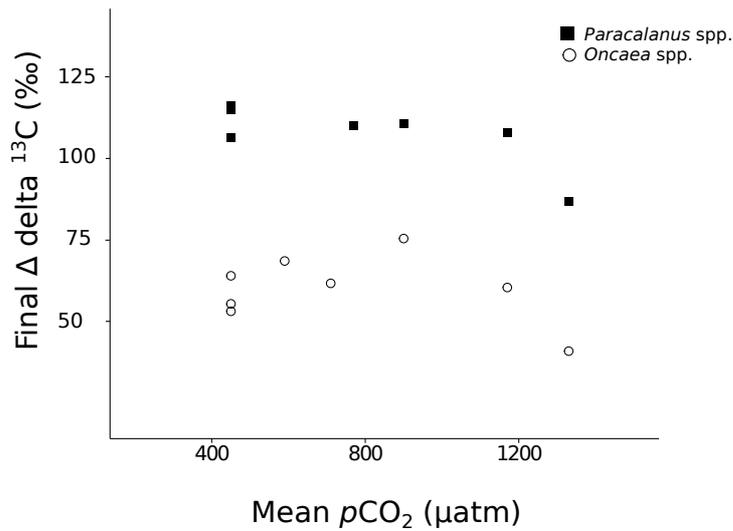


Figure III-8. Final isotopic signature ($\Delta\delta^{13}\text{C}$ in ‰) of the zooplankton species *Paracalanus* spp. and *Oncaea* spp. as a function of average $p\text{CO}_2$ levels in each mesocosm over the experimental period, during the experiment conducted in the Bay of Calvi in summer 2012.

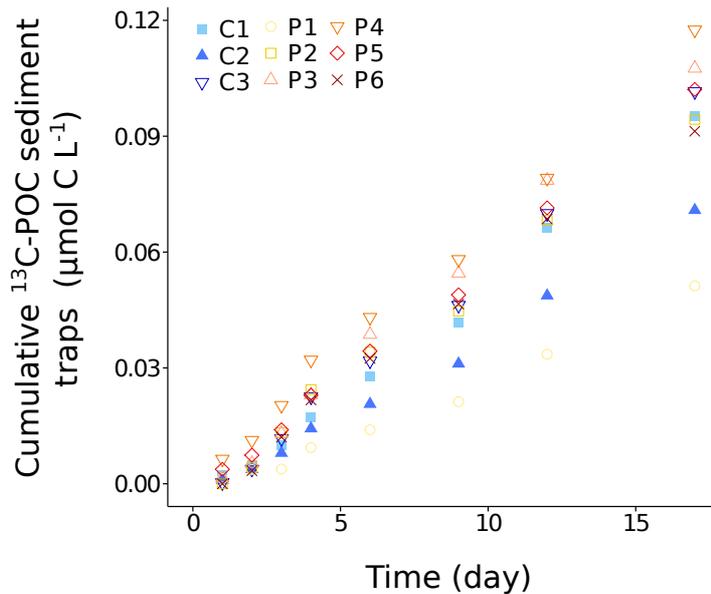


Figure III-9. Cumulative ^{13}C enrichment of the sediment trap organic particles in all mesocosms (C1 to P6) during the experiment conducted in the Bay of Calvi.

Table III-2. Cumulative production ($\mu\text{mol C L}^{-1}$) in all mesocosms (C1 to P6) of fast incorporating phytoplankton species (autotrophs), slow incorporating phytoplankton species (mixotrophs) and bacteria during the experiments in the Bay of Calvi (BC) and in the Bay of Villefranche (BV). Mean $p\text{CO}_2$ value for the period considered (μatm).

	C1	C2	C3	P1	P2	P3	P4	P5	P6
BC									
Mean $p\text{CO}_2$ (μatm)	429	427	429	508	586	660	747	828	990
Autotrophs	0.03	ND	0.01	0.01	0.03	-0.01	0.02	0.02	-0.01
Mixotrophs	0.10	0.04	0.07	0.04	0.12	0.07	0.10	0.02	0.02
Bacteria	0.02	0.004	0.02	0.02	0.04	0.01	0.006	0.02	0.02
BV									
Mean $p\text{CO}_2$ (μatm)	356	351	346	469	516	591	606	824	1095
Autotrophs	1.00	0.76	1.24	0.89	0.95	1.04	0.68	1.03	0.66
Mixotrophs	0.66	0.53	0.90	0.53	0.68	0.87	0.60	0.98	0.49

2.4 Discussion

Carbon-13 enrichment was very successful with a good incorporation into pelagic particulate organic matter and subsequent transfer to bacteria and zooplankton as well as export to sediment traps. The ^{13}C incorporation in all compartments of the plankton communities investigated allowed a quantitative and qualitative description of the dynamics of these communities. The two study sites presented different initial characteristics with summer stratified in BC and pre-bloom conditions in BV, although both being characterized by low nutrient and low chlorophyll *a* levels ($\text{chl } a < 1.3 \mu\text{g chl } a \text{ L}^{-1}$; see Gazeau et al., in prep, a and Chapter II of the thesis). In chapter II, metabolic rates results have shown a near metabolic equilibrium (oscillating around $0 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) in BC while in BV the systems clearly tended to the autotrophic state. The net community and group-specific production rates determined in this chapter also support these findings, although the temporal dynamics were not always similar between methods. Indeed, in BV, NCP- ^{13}C decreased while NCP measured with the oxygen light-dark technique (NCP- O_2) increased during the course of the experiment with a NCP- O_2 evolving toward a more autotrophic system and no clear trend could be observed based on the ^{14}C technique (see chapter II). These measurements were obtained through small volume incubations over 24 h that seem to have favoured phytoplankton production, potentially by presenting more nutrient availability at the cells surroundings or modifying seawater movement and irradiance. In BC, autotrophs and mixotrophs production rates and NCP- ^{13}C have shown an increase till day 10 that is consistent with gross primary production rates measured with the oxygen light-dark method. This increase was associated to a stratification event and an increase in *Synechococcus* spp. abundance (see chapter II). Nevertheless, NCP- ^{13}C measured directly in the mesocosm were more elevated in BV than in BC suggesting that the community in BV was more autotrophic. In addition, in BC, mixotroph production and biomass exceeded the autotrophs ones suggesting an efficient nutrient recycling to support phytoplankton production even under very low nutrients levels. In BV, autotrophs and mixotrophs biomasses were almost similar while autotroph production rates exceeded mixotroph production, suggesting that the ecosystem was more based on new production rather than regenerated production.

These mesocosm ^{13}C labelling studies are the first to be performed under maintained low nutrient conditions and with low phytoplankton biomass. The restricted sampling volume (~ 4 L) due to sampling protocol, made the determination and quantification of PLFA difficult. However, PLFA remained useful tools to understand the functioning of an ecosystem in

particular when combined with stable isotope analysis (Middelburg, 2014). Following De Kluijver et al. (2013), the conservative approach using large phytoplankton groups based on incorporation pattern were used to obtain taxa-specific groups. This was motivated in our study by the very low concentrations of PLFA. Although PLFA are good taxonomic markers, most are shared by several phytoplankton groups and as in the Mediterranean Sea, very few studies have been conducted on PLFA and, as the PLFA composition of each species present should be known (Zelles, 1999) to avoid misinterpretation, it was therefore decided to link groups of specific PLFA to taxa. Moreover, conversion factors used in this study to estimate carbon biomass from PLFA concentrations were based on the analysis of phytoplankton strains sampled in estuaries and productive areas or cultures under replete nutrient conditions. This certainly introduced some errors in the estimates of absolute biomass and production reported here. Conversion factors are used as indicators and because the uncertainty resulting from their use is expected to occur similarly in all mesocosms, thus they do not explain the potential effect of elevated CO₂.

The different net community production, phytoplankton group-specific and bacteria production rates as well as PLFA based biomasses obtained during the two experiments did not show any relationship with increasing *p*CO₂ levels. This is fully in agreement with the absence of CO₂ effect reported in chapter II on primary production rates measured by other incubation methods (O₂ light-dark and ¹⁸O, ¹⁴C labelling). Biomasses based on PLFA concentrations have not shown any effect of ocean acidification that is consistent with pigment analyses, phytoplankton cell counts (Gazeau et al., in prep, b) and bacterial abundances (Celussi et al., in prep). Despite differences in absolute biomass concentration, biomasses of phytoplankton based on PLFA and chlorophyll *a* have shown similar temporal dynamic. The same dynamic but different absolute value were also observed for bacteria abundances based on PLFA and flow cytometry. In addition to the uncertainties linked to the use of inappropriate conversion factors, bacterial PLFA take into account free-living and attached bacteria while flow cytometry takes into account only free-living bacteria, potentially explaining the important differences observed between the two methods. The zooplankton isotopic signature at the end of the experiment in BC did not show any *p*CO₂ effect albeit high CO₂ levels tended to have lower Δδ¹³C for both species collected and would correspond to lower zooplankton grazing in these mesocosms. Sediment traps were placed at 12 m and therefore are not representative of a real export below the euphotic zone. They were also in the daily migration depth of some zooplankton and despite most of the swimmers were removed they can contribute for a large fraction of settling material in terms of weight and

organic carbon content. Nevertheless, freshly exported particulate matter in the BC was not sensitive to increase CO₂ and is consistent with the fact that no effect was measured in total and group-specific production rates as well as with the fact that transparent exopolymeric particles (TEP) were not affected by increased CO₂ levels (Luculano et al., in prep).

To date, only one mesocosm experiment has been performed following the same set-up (50 m³ off-shore mesocosms) and ¹³C enrichment protocol (De Kluijver et al., 2013). During this experiment in Arctic waters (hereafter called Svalbard), the effects of ocean acidification on production rates and carbon fluxes were subtle and differed following the phase considered (before vs after nutrient addition). During this first 12 days, nutrient (nitrogen as nitrate and nitrite as well as phosphate) concentrations were close to or below detection limits of the conventional methods used suggesting there were low similarly to our experiments. Although chlorophyll *a* concentrations were similar between the experiment in BV and in Svalbard, POC concentrations were 2 to 3 times higher in Svalbard (~ 20-30 μmol L⁻¹; Schulz et al., 2013) than in BV (~10 μmol L⁻¹). The phytoplankton communities were composed of small species with haptophytes in all three experiments (BC, BV and Svalbard; Schulz et al., 2013; Gazeau et al., in prep, b) and communities differed by the presence of other small species such as cyanobacteria (mostly *Synechococcus* spp.) in BC and pelagophytes in BV that were absent or not reported as such in Svalbard where nano- and pico-phytoplankton were reported (Brussaard et al., 2013). It is now believed that plankton community response to ocean acidification depends on environmental conditions and a recent study has highlighted the preponderant role of the community structure to respond to ocean acidification (Eggers et al., 2014). Phytoplankton species have several carbon concentration mechanisms (CCMs), which efficiencies differ among species (e.g. Rost et al., 2008; Reinfelder, 2001). Therefore increasing CO₂ levels could benefit to some species that could down-regulate CCM activities and save energy to enhance primary production. The initial ratio of diatoms, dinoflagellates and cyanobacteria could thus be responsible for large differences in the response to ocean acidification (Eggers et al., 2014). Our results suggest that natural assemblages with larger proportion of haptophytes, cyanobacteria (mostly *Synechococcus* spp.) and other small phytoplankton species will be insensitive to ocean acidification in terms of primary production and biomass. Similarly, in Svalbard, NCP-¹³C as well as NCP-O₂ did not change with *p*CO₂ but group-specific production rates have shown different responses with enhanced and decreased production rates for autotrophs and mixotrophs, respectively. Comparing these three large mesocosm experiments comfort the idea that small differences in plankton communities can lead to different responses to ocean acidification. However the

differences cannot be measured using common methods of metabolic rates measurement despite the different responses within the community and analyses of labelled ^{13}C -PLFA have proven to be an appropriate tool. Therefore global primary production does not reflect the ecological response due to inadequate methods and ocean acidification could have more influence on plankton community composition than on primary production (Hein and Sand-Jensen, 1997).

The fact that no effect of ocean acidification was detected in the two experiments performed at two locations and seasons in the NW Mediterranean Sea for the different parameters measured (production rates, biomasses, grazing and export) is very coherent. Considering mesocosms are representative of natural conditions, our findings suggest that ocean acidification would have a limited effect on plankton community structure and carbon transfer within pelagic compartments in oligotrophic areas. In addition, the different responses obtained between the two oceanic provinces compared (Arctic vs Mediterranean Sea) shows the necessity to have a regional approach while studying the biological response to climate change (Häder et al., 2014). Temperature, nutrient availability, plankton community composition and other unidentified parameters are major environmental and biological aspects that control the effect of human-induced perturbations such as ocean acidification.

Chapter IV

Combined effects of temperature and $p\text{CO}_2$ increase on a plankton community



Effect of ocean warming and acidification on a plankton community in the NW Mediterranean Sea

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The effect of ocean warming and acidification was investigated on a natural plankton assemblage from an oligotrophic area, the bay of Villefranche (NW Mediterranean Sea). The assemblage was sampled in March 2012 and exposed to the following four treatments for 12 days: control ($\sim 360 \mu\text{atm}$, 14°C), elevated $p\text{CO}_2$ ($\sim 610 \mu\text{atm}$, 14°C), elevated temperature ($\sim 410 \mu\text{atm}$, 17°C), and elevated $p\text{CO}_2$ and temperature ($\sim 690 \mu\text{atm}$, 17°C). Nutrients were already depleted at the beginning of the experiment and the concentrations of chlorophyll *a* (chl *a*), heterotrophic prokaryotes and viruses decreased, under all treatments, throughout the experiment. There were no statistically significant effects of ocean warming and acidification, whether in isolation or combined, on the concentrations of nutrients, particulate organic matter, chl *a* and most of the photosynthetic pigments. Furthermore, ^{13}C labelling showed that the carbon transfer rates from ^{13}C -sodium bicarbonate into particulate organic carbon were not affected by seawater warming nor acidification. Rates of gross primary production followed the general decreasing trend of chl *a* concentrations and were significantly higher under elevated temperature, an effect exacerbated when combined to elevated $p\text{CO}_2$ level. In contrast to the other algal groups, the picophytoplankton population (cyanobacteria, mostly *Synechococcus*) increased throughout the experiment and was more abundant in the warmer treatment though to a lesser extent when combined to high $p\text{CO}_2$ level. These results suggest that under nutrient-depleted conditions in the Mediterranean Sea, ocean acidification has a very limited impact on the plankton community and that small species will benefit from warming with a potential decrease of the export and energy transfer to higher trophic levels.

Keywords: climate change, ocean acidification, ocean warming, oligotrophic area, plankton community, primary production.

Introduction

Anthropogenic carbon dioxide (CO_2) emissions are responsible for an important increase in atmospheric CO_2 partial pressure ($p\text{CO}_2$). The consequences of CO_2 emissions are an increase of surface ocean temperature expected to rise by $2\text{--}4^\circ\text{C}$ by the end of this century based on the current emission rates (IPCC, 2013). About 25% of anthropogenic CO_2 emissions are absorbed by the ocean (Le Quéré *et al.*, 2013), generating profound modifications of the ocean carbonate chemistry and referred to as “ocean acidification”. The pH of the surface ocean has decreased by 0.1 units since the beginning of the industrial era and is projected to decrease by an extra 0.3–0.4 units by the end of the present century (Orr, 2011). Seawater

warming and acidification are expected to significantly affect the carbon cycle through the changes in the functioning of marine organisms and communities.

CO_2 fluxes between the atmosphere and the ocean are partly driven by biological activity. In the surface mixed layer, the balance between the autotrophic fixation of CO_2 by primary producers and the consumption/mineralization of organic matter by the whole plankton community is referred to as the net community production (NCP). A system is referred to as autotrophic when production exceeds consumption and heterotrophic when consumption is higher than production. Depending on the atmospheric $p\text{CO}_2$ and sea surface temperature, the surface ocean potentially acts

as a sink of CO₂ for the atmosphere (Gattuso *et al.*, 1998) when the surface mixed layer exports organic matter to the deep ocean. Primary producers have then a key role on carbon cycle and climate regulation.

In many marine plants and algae, RuBisCO, a key enzyme involved in CO₂ fixation, is generally limited at environmental CO₂ concentrations. An increase in CO₂ could therefore enhance phytoplankton photosynthesis and growth (Riebesell *et al.*, 2007). However, experimental studies reported contrasting effects of elevated CO₂ on photosynthesis: stimulating, neutral or even inhibitory effects were found (see review by Riebesell and Tortell, 2011 and references therein). Such different responses could be due to species-specific differences in the efficiency of carbon concentrating mechanisms (CCMs; e.g. Giordano *et al.*, 2005). Depending on the efficiency of their CCM, some species will benefit from elevated CO₂ conditions while others will not, provoking changes in community composition. Shifts toward smaller (e.g. Yoshimura *et al.*, 2010) or larger phytoplankton cells (Tortell *et al.*, 2002) have been observed while no change in the taxonomic composition have been reported for communities acclimated to large seasonal pH changes (Nielsen *et al.*, 2012). Changes in community composition could have consequences on ecological processes (such as modifications of energy transfer to higher trophic levels) and biogeochemical cycling (i.e. modifications of the export to the deep ocean; Riebesell *et al.*, 2007). With respect to ecosystem function, several studies have shown enhanced carbon fixation and an increase in the organic carbon to nitrogen ratio (e.g. Riebesell *et al.*, 2007), while others reported limited or no effect (Feng *et al.*, 2009).

Projected warming is also expected to significantly affect marine organisms and communities. Culture experiments and *in situ* sampling have shown increased metabolic rates as temperature increases (e.g. Eppley, 1972; Regaudie-de-Gioux and Duarte, 2012). However, as thermal tolerance greatly differs between species, some will face conditions outside of their tolerance range and will be forced to move their ecological niches (Gao *et al.*, 2012 and references therein). Several experiments have shown that warming could induce a shift towards smaller phytoplankton species (Sommer and Lengfellner, 2008) as well as a tighter coupling between phytoplankton and bacteria with possible consequences on remineralization and carbon export (Hoppe *et al.*, 2008). Furthermore, phytoplankton exhibits higher nitrogen to phosphate requirements in warmer conditions (Toseland *et al.*, 2013), which might also impact biogeochemistry.

In recent years, an experimental effort has been initiated to investigate the effect of both drivers at the community level. Elevated temperature combined or not with elevated pCO₂ has been shown to enhance photosynthetic rates (Hare *et al.*, 2007; Feng *et al.*, 2009) as well as enhanced dissolved organic carbon relative to particulate organic production (Kim *et al.*, 2011). A recent study focusing on the short- (2 weeks) and long-term (1 year) response of a diatom community showed that elevated pCO₂ and temperature, whether combined or taken in isolation, had an effect on the community structure, with a stronger influence of warming which induces a loss in species richness (Tatters *et al.*, 2013). Another study supported the predominant effect of warming compared with acidification on bacterial phylogenetic composition (Lindh *et al.*, 2013).

Nutrient availability is suspected to also have strong effects on the community response to ocean warming and acidification (Hare *et al.*, 2007) and the great majority of past experiments have been performed under nutrient replete conditions. However, a large part (>60%) of the open ocean is characterized by oligotrophic

conditions with very low nutrient concentrations and rates of primary production (Dodds and Cole, 2007). Although the metabolic status (auto- vs. heterotrophic) of these areas is still under debate (Duarte *et al.*, 2013; Williams *et al.*, 2013) oligotrophic provinces represent ~30% of global oceanic primary production (Longhurst *et al.*, 1995). Therefore, changes in the community composition and functioning in these regions could lead to significant changes in the global oceanic CO₂ sink. The Mediterranean Sea is a largely enclosed sea, presenting trophic status varying from mesotrophic in the Northwestern region to extremely oligotrophic in the Eastern basin. Despite these environmental constraints, the Mediterranean Sea hosts from 4 to 18% of the Earth's marine biodiversity (Bianchi and Morri, 2000) with a high percentage of endemic species. There is a growing concern on the effects of climate change and ocean acidification in this area, although, to the best of our knowledge, no experiment on the effect of elevated temperature and pCO₂ on natural plankton communities have been conducted to date.

In the present study, a Mediterranean plankton community sampled in winter was exposed to elevated temperature and pCO₂ as projected for the end of the century (respectively, +3°C and ×2 pCO₂). During 12 days, experimental bottles were placed in a control and a temperature-regulated outdoor tank. Parameters and processes such as carbonate chemistry, nutrients, particulate organic matter, pigments, cells abundance, and primary production were monitored regularly. Stable carbon isotope tracers (¹³C) were also used to measure carbon fixation.

Material and methods

Experimental setup

A volume of 300 l of seawater was sampled in the bay of Villefranche (France; 43°40'N, 7°18'E) at 5 m on 14 March 2012. Pumping was performed by a trace-metal clean pump activated by pressurized air from a diving tank, preventing any damage on the organisms. Seawater was sieved onto a 200-μm mesh to remove large organisms.

In the laboratory, seawater was transferred to an acid-cleaned 300 l tank. Labelled ¹³C-sodium bicarbonate was added to a final concentration of 19 μmol l⁻¹ corresponding to 0.83% of total dissolved inorganic carbon (DIC) concentration and increasing δ¹³C-DIC by 760‰. A first set of 4 l acid-cleaned polycarbonate (PC) bottles (*n* = 24) was filled and hermetically sealed. CO₂-saturated filtered seawater was gently mixed with the remaining water to reach a calculated pCO₂ of ~750 μatm. This elevated pCO₂ seawater was then distributed to 4 l acid-cleaned polycarbonate bottles (*n* = 24). Half of the ambient and elevated pCO₂ bottles were placed in a 2 m³ tank installed on the pier of the Laboratoire d'Océanographie de Villefranche with a continuous flow of *in situ* seawater (20 l min⁻¹). The other half was placed in another identical tank in which temperature was maintained at ca. 3°C above *in situ* temperature. The four treatments, including three replicates bottles per sampling day, were: Control (C; ambient pCO₂ and temperature), Ocean Warming (OW; ambient pCO₂ and elevated temperature), Ocean Acidification (OA; elevated pCO₂ and ambient temperature), and Greenhouse (G; elevated pCO₂ and temperature). The bottles were gently stirred every day to keep particles suspended. Light (natural sunlight) conditions were similar between the different treatments and were representative of surface conditions (~1 m depth). On several occasions, photosynthetic active radiation (PAR) was measured in the tanks with a spherical sensor

connected to an LICOR data logger, and daily (sunrise–sunset) averages ranged between 1025 and 1213 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Temperature was measured with a Seabird SBE37 temperature sensor and temperature in the elevated temperature tank was regulated using a COREMA[®] controller. Temperature regulation was performed throughout the experiment but, due to a technical problem, data were not logged on the first day.

Sampling and analyses

After 2, 4, 8, and 12 days (thereafter referred to as d2, d4, d8, and d12), three bottles of each treatment were removed from the tanks. Samples for DIC were stored in 60 ml brown borosilicate bottles and poisoned with 10 μl saturated solution of mercuric chloride (HgCl_2). For total alkalinity (A_T) determination, 500 ml was filtered through 47 mm GF/F filter, poisoned with HgCl_2 and preserved at 4°C pending analysis. Aliquots of 20 ml for the determination of inorganic phosphate (PO_4^{3-}), nitrate and nitrite ($\text{NO}_x = \text{NO}_3^- + \text{NO}_2^-$) were filtered through 0.2 μm polycarbonate filters cleaned with Merck Suprapur hydrochloric acid and rinsed with 18.2 $\text{M}\Omega \text{ cm}^{-1}$ ultra pure water. Samples were stored in 125 ml polyethylene (PE-HD) flasks, cleaned with Merck Suprapur hydrochloric acid, and acidified with Merck Ultrapur HCl, and finally processed using a spectrophotometric method with a Liquid Waveguide Capillary Cell optic fibre, allowing the determination of nanomolar concentrations phosphate and NO_x with detection limits of 1 and 9 nmol l^{-1} , respectively, and a precision (coefficient of variation) of $\sim 7\%$ for both parameters (Adornato *et al.*, 2007; Zimmer and Cutter, 2012). Samples for the determination of silicate were stored in acid-cleaned PE vials, poisoned with a saturated solution of HgCl_2 and kept at 4°C pending analysis using an AXFLOW AA3 auto-analyzer. A 2 ml aliquot of seawater was added to 80 μl of glutaraldehyde for the analysis of community composition. Samples were snap-frozen in liquid nitrogen and kept at -80°C pending analysis on a FACSCalibur flow cytometer. For pigment analyses, 0.5–1 l of seawater were filtered on 25 mm GF/F membranes which were stored at -80°C pending extraction and analysis on an Agilent Technologies 1200 series following the protocol of Ras *et al.* (2008). For particulate organic carbon (POC) concentration and its isotopic signature ($\delta^{13}\text{C}$ -POC), 1 l of seawater was filtered through pre-combusted and preweighted 25 mm GF/F filters under low pressure. Filters were dried at 60°C for 24 h then stored at room temperature in the dark. For measurement of $\delta^{13}\text{C}$ -DIC, samples (20 ml) were poisoned with 10 μl of HgCl_2 and stored at room temperature in the dark. The remaining seawater in each 4 l polycarbonate bottle was used for measurements of community metabolism (see below).

DIC was determined immediately after opening the bottles 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 before sample analysis against a certified reference material provided by A. Dickson (Scripps Institution of Oceanography, San Diego, CA, USA; batch 114). The average precision (*SD*) of all measurements ($n = 52$; ran in triplicates) was 0.7 $\mu\text{mol kg}^{-1}$. A_T was determined on triplicate 50 ml subsamples by potentiometric titration on a Metrohm Titrando 80 titrator coupled to a glass electrode (Metrohm, electrode plus) and a thermometer. The pH electrode was calibrated daily on the total scale using TRIS buffers of salinity 35 provided by A. Dickson. Measurements were carried out at 25°C and A_T was calculated as described by Dickson *et al.* (2007). Along the experiment, standards provided by A. Dickson (batch 108)

were used to check precision and accuracy ($n = 18$; 2.9 and 3.7 $\mu\text{mol kg}^{-1}$, respectively). The parameters of the carbonate system were determined from DIC, A_T , temperature, and salinity using the R package seacarb (Lavigne *et al.*, 2014). To take into account the uncertainty of the measured input parameters during the calculation of the carbonate chemistry parameters, a Monte-Carlo procedure was applied. One thousand values were randomly chosen between the mean $\pm SD$ of each measured parameter and mean $\pm SD$ of each computed parameters is reported.

POC samples were analysed for organic carbon content and isotope ratios on an elemental analyser (Thermo Electron Flash EA 1112) coupled to a Delta V isotope ratio mass spectrometer (IRMS). For DIC isotope analyses, a 2 ml helium headspace was created in the vials and samples were acidified with 2 μl of phosphoric acid (H_3PO_4 ; 99%). After equilibration for 30 min, the CO_2 concentration and its isotopic composition in the headspace were measured on the EA-IRMS.

Data of the ^{13}C -labelling study were expressed in the delta notation (δ) relative to Vienna Pee Dee Belemnite standard. The carbon isotope ratio was calculated as

$$R_{\text{sample}} = \left(\frac{\delta^{13}\text{C}_{\text{sample}}}{1000 + 1} \right) \times R_{\text{VPDB}}, \quad \text{with } R_{\text{VPDB}} = 0.0111797.$$

The ^{13}C fraction was calculated as:

$$^{13}\text{F} = \frac{^{13}\text{C}}{^{13}\text{C} + ^{12}\text{C}} = \frac{R}{R + 1},$$

where $R = ^{13}\text{C}/^{12}\text{C}$.

The excess ^{13}C was obtained as $\Delta^{13}\text{F} = ^{13}\text{F}_{\text{sample}} - ^{13}\text{F}_{\text{background}}$. Absolute incorporation rates were calculated as ^{13}C -POC = $\Delta^{13}\text{F} \times [\text{POC}]_{\text{sample}}$ ($\mu\text{mol C l}^{-1}$; De Kluijver *et al.*, 2010).

Finally, ^{13}C -concentrations were converted to total fresh POC:

$$\text{New-POC} = \left(\frac{\Delta^{13}\text{F}_{\text{POC}}}{\Delta^{13}\text{F}_{\text{DIC}}} \right) \times [\text{POC}],$$

where $\Delta^{13}\text{F}_{\text{POC}}$ and $\Delta^{13}\text{F}_{\text{DIC}}$ are the excess values, [POC] is a concentration in $\mu\text{mol C l}^{-1}$.

NCP is then calculated as:

$$\text{NCP-}^{13}\text{C} = \frac{\Delta\text{New-POC}}{\Delta t},$$

where $\Delta\text{New-POC}$ is the differences between two consecutive sampling days.

Primary production and community respiration

NCP (NCP- O_2) and community respiration (CR- O_2) were measured using the oxygen light–dark technique. Gross community production was measured using the ^{18}O -labelling method (gross primary production, GPP- ^{18}O).

Before sunrise, three 60 ml biological oxygen demand bottles were sampled from each PC bottle. One bottle was immediately fixed with Winkler reagents to determine the initial O_2 concentration. A transparent and a dark bottle were incubated in the outdoor tanks for 24 h for estimating NCP- O_2 and CR- O_2 , respectively. O_2 concentrations were measured using an automated Winkler titration technique with potentiometric endpoint detection. Analyses were performed with a Metrohm Titrando 888 with

a Metrohm ion electrode. Reagents and standardizations were similar to those described by Knap *et al.* (1996). NCP-O₂ and CR-O₂ were estimated by regressing O₂ values against time, and CR was expressed as negative values. GPP (GPP-O₂) was calculated as the difference between NCP-O₂ and CR-O₂. The combined errors were calculated as:

$$SE_{x-y} = \sqrt{SE_x^2 + SE_y^2}$$

For the ¹⁸O-labelling technique, samples were transferred from each PC bottle to two 60 ml transparent glass bottles and sealed. One bottle was directly poisoned with 10 μl saturated solution of HgCl₂ to estimate the natural isotopic composition and the other bottle was spiked, with 50 μl of 97% H₂¹⁸O to reach a final isotopic composition δ¹⁸O-H₂O of 335‰. After 12 h incubations in the outdoor tank (from sunrise to sunset), samples were poisoned using HgCl₂ and stored upside down in the dark at room temperature pending analysis. Measurements were performed at KU Leuven (Belgium). A headspace of 3 ml was created with helium and allowed to equilibrate for 30 min (¹⁸O-O₂ measurements). The extracted water was injected into helium-flushed vials (¹⁸O-H₂O measurements). Pure CO₂ (100 μl) was then added and samples were allowed to equilibrate for 24 h. δ¹⁸O-H₂O was therefore measured as δ¹⁸O-CO₂. Determinations of δ¹⁸O-O₂ and δ¹⁸O-CO₂ were accomplished using an elemental analyzer (Flash HT/EA) coupled to a Delta V IRMS. An overflow technique was used to limit air contamination of the needle. For δ¹⁸O-O₂, the internal standard used to correct the data and survey instrumental deviation was air from the outside. For δ¹⁸O-CO₂, a calibration was performed with a VSMOW standard. GPP rates (μmol O₂ l⁻¹ d⁻¹) were calculated using the following equation (Kiddon *et al.*, 1995):

$$GPP-^{18}O = \left[\frac{\delta^{18}O-O_2 \text{ final} - \delta^{18}O-O_2 \text{ init}}{\delta^{18}O-H_2O - \delta^{18}O-O_2 \text{ init}} \right] \times [O_2]_{\text{init}}$$

where δ¹⁸O-O₂ init and δ¹⁸O-O₂ final are measured δ¹⁸O-O₂ before and after incubation (‰), respectively, δ¹⁸O-H₂O is the final isotopic composition of the labelled water (‰), and [O₂]_{init} is the O₂ concentration before incubations (μmol l⁻¹). The overall error was estimated using a Monte-Carlo procedure as described earlier.

Statistics and data availability

Data are presented as averages ± SD (or ± SE for metabolic rates). Due to the small number of replicates (×3), PERMANOVA analyses were performed using the R package RVAideMemoire (Hervé, 2013) to test for differences in parameters/processes between the four different treatments. These analyses were performed considering two interacting factors (*p*CO₂ and temperature) and one blocking factor (time) over 1000 permutations and a significant effect was considered when *p* < 0.05. Cumulative metabolic rates were calculated for the whole experimental period. Values for days when no incubations were performed were obtained by linear interpolation and the cumulative values were then summed up for the experimental period. The data reported here as well as complementary parameters are freely available in Pangaea: <http://doi.pangaea.de/10.1594/PANGAEA.834159>

Results

In the two tanks, temperature naturally varied by ~2°C between day and night (Figure 1). The natural average temperature was 14.3 ± 0.3°C while it was on average 17.2 ± 0.7°C in the elevated temperature tank (average difference: 2.8°C). In treatments C and OW, *p*CO₂ was on average 364 ± 14 μatm (pH_T 8.12 ± 0.02) and 414 ± 12 μatm (pH_T 8.07 ± 0.01), respectively. Elevated *p*CO₂ conditions were on average 613 ± 22 μatm (pH_T 7.92 ± 0.01) and 690 ± 28 μatm (pH_T 7.88 ± 0.02) for treatments OA and G, respectively. The targeted *p*CO₂ levels were not reached most likely as a consequence of significant outgassing while bottles were filled. A_T averaged for all treatments was 2568 ± 4 μmol kg⁻¹ (Table 1) and did not vary significantly between treatments and sampling days (Table 2).

All dissolved inorganic nutrients were close to the detection limit. The concentration of NO_x and silicate did not vary between treatments (Table 2; Figure 2a and b) but the phosphate concentration was significantly lower under elevated *p*CO₂ (*F* = 13.19, *p* < 0.05; Table 2, Figure 2c). Phosphate was on average 13 ± 1 nmol P l⁻¹ and NO_x (NO₃⁻ + NO₂⁻) concentrations remained constant (75 ± 20 nmol N l⁻¹), after an initial decrease between d0 and d2 (Figure 2b). Silicate concentrations did not vary with time and averaged 1.0 ± 0.1 μmol Si l⁻¹ (Figure 2a).

The concentration of POC did not significantly differ between treatments (Table 2) with an overall mean of 11 ± 1 μmol C l⁻¹ (Figure 3a). PON concentrations were low (mean: 0.8 ± 0.1 μmol N l⁻¹), except on d2 in the OW and OA treatments where concentrations reached 1.9 ± 0.4 μmol N l⁻¹ (Figure 3b). The particulate organic C : N ratio was high with a global average of 15 ± 1 (6 ± 1 for OW and OA on d2) and was not different between treatments (Table 2). As for POC and PON, the concentration of chlorophyll *a* (chl *a*) did not differ between treatments (Table 2) but varied significantly throughout the experiment (Figure 3c). It increased from an overall mean of 0.9 ± 0.1 μg l⁻¹ on d0 to 1.1 ± 0.0 μg l⁻¹ on d2. After d2, it decreased in all treatments to reach an average final concentration of 0.3 < 0.1 μg l⁻¹.

In terms of phytoplankton group succession during the experiment, diatoms (as represented by fucoxanthin pigments), prymnesiophytes (19'-hexanoyloxyfucoxanthin), and cyanobacteria (zeaxanthin) were detected in the samples with a large dominance

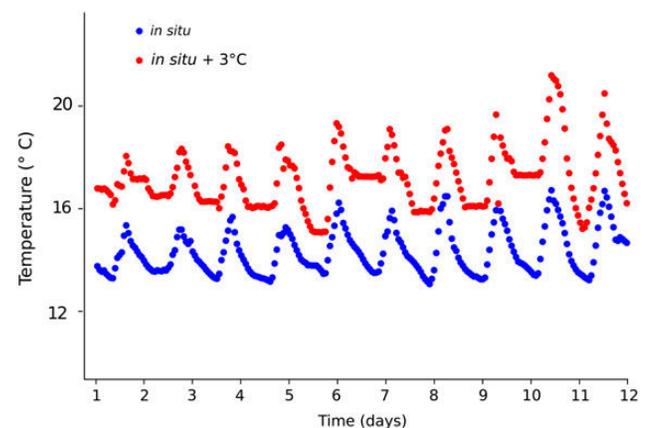


Figure 1. Temperature during the experiment. Blue: *in situ* temperature (Control and Ocean Acidification treatments). Red: *in situ* + 3°C (Ocean Warming and Greenhouse treatments).

Table 1. Carbonate chemistry parameters in the control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments (average \pm SD).

	Alkalinity ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	$p\text{CO}_2$ (μatm)	pH_T
Day 0				
C	2566 \pm 1	2278 \pm 0	366 \pm 2	8.11 < 0.01
OW	2567 \pm 2	2286 \pm 0	428 \pm 1	8.05 < 0.01
OA	2570 \pm 2	2386 \pm 0	622 \pm 3	7.91 < 0.01
G	2565 \pm 1	2352 \pm 0	618 \pm 2	7.92 < 0.01
Day 2				
C	2566 \pm 2	2283 \pm 2	377 \pm 2	8.10 < 0.01
OW	2563 \pm 0	2284 \pm 2	431 \pm 5	8.05 < 0.01
OA	2565 \pm 1	2371 \pm 6	586 \pm 18	7.94 \pm 0.01
G	2566 \pm 4	2374 \pm 2	666 \pm 2	7.89 < 0.01
Day 4				
C	2566 \pm 2	2270 \pm 3	356 \pm 6	8.12 < 0.01
OW	2566 \pm 2	2273 \pm 1	405 \pm 1	8.07 < 0.01
OA	2568 \pm 2	2383 \pm 3	620 \pm 17	7.92 \pm 0.01
G	2566 \pm 3	2386 \pm 3	716 \pm 22	7.86 \pm 0.01
Day 8				
C	2568 \pm 5	2277 \pm 4	363 \pm 12	8.12 \pm 0.01
OW	2567 \pm 1	2279 \pm 1	415 \pm 1	8.06 < 0.01
OA	2567 \pm 1	2385 \pm 4	629 \pm 13	7.91 < 0.01
G	2573 \pm 3	2380 \pm 2	669 \pm 14	7.89 < 0.01
Day 12				
C	2577 \pm 11	2281 \pm 6	359 \pm 23	8.12 \pm 0.02
OW	2569 \pm 1	2275 \pm 3	404 \pm 5	8.07 < 0.01
OA	2568 \pm 0	2383 \pm 2	617 \pm 4	7.92 < .01
G	2569 \pm 5	2387 \pm 8	708 \pm 22	7.87 \pm 0.01
Average				
C	2569 \pm 5	2278 \pm 5	364 \pm 14	8.12 \pm 0.02
OW	2566 \pm 2	2279 \pm 6	414 \pm 12	8.07 \pm 0.01
OA	2568 \pm 2	2382 \pm 6	613 \pm 22	7.92 \pm 0.01
G	2568 \pm 3	2377 \pm 12	690 \pm 28	7.88 \pm 0.02

Total alkalinity (A_T) and dissolved inorganic carbon (DIC) were measured, while the partial pressure of CO_2 ($p\text{CO}_2$) and pH_T were estimated based on DIC and A_T using seacarb (see the Material and method for more details).

of prymnesiophytes (28% on average; Figure 4a–c). Variations in the concentration of diatoms and prymnesiophytes were similar to chl *a* variations, i.e. an increase during the first 2 days followed by a general decrease. Variations in zeaxanthin concentrations indicate that the abundance of cyanobacteria increased from d0 to d8 then declined at the end of the experiment. This is consistent with flow cytometer data which show the same dynamics for the cyanobacteria *Synechococcus* (Figure 4f) with a significant correlation between the two techniques (HPLC and flow cytometry; $r = 0.86$, $p < 0.01$, $n = 52$). The abundance of *Prochlorococcus*, another cyanobacteria, increased until d4 then decreased (data not shown). Pigments and flow cytometry data showed a specific response to elevated temperature alone for cyanobacteria (zeaxanthin: $F = 6.98$, $p < 0.05$; *Synechococcus* by flow cytometry: $F = 6.11$, $p < 0.05$; Table 2, Figure 4c and f). The abundance of pico-eukaryotes was significantly different at elevated $p\text{CO}_2$ ($F = 8.69$, $p < 0.05$; Table 2), a difference that could be attributed to a transient higher abundance of this group in the OA treatment on d2 (data not shown). The abundance of viruses and heterotrophic prokaryotes decreased with time (Figure 4d and e). There was no difference between treatments for heterotrophic prokaryotes (Table 2, Figure 4d) and a significant temperature effect was found for viruses ($F = 5.57$, $p < 0.05$; Table 2) due to two very high values

in the C and OA treatments on d8 (Figure 4e), this effect disappeared when these two values were omitted.

Based on the O_2 light–dark technique, no significant difference between treatments was observed for the considered metabolic processes (NCP- O_2 , GPP- O_2 , and CR- O_2 ; Table 2). NCP- O_2 ranged from -1.6 ± 0.8 to $2.8 \pm 0.2 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$ while CR- O_2 ranged from -4.3 ± 0.8 to $-0.8 \pm 1.3 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$ (Figure 5a and b). GPP- O_2 varied from -0.15 ± 1.5 to $5.74 \pm 3.1 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$ (Figure 5c). NCP- O_2 did not show a clear temporal trend except for G treatment for which NCP- O_2 decreased from autotrophic to heterotrophic conditions throughout the experiment and in general, very large variations were observed for all treatments (Figure 5a). GPP- ^{18}O followed the decreasing trend of chl *a* (Figure 5d) and was significantly increased under elevated temperature ($F = 15.82$, $p < 0.01$; Table 2) with a significant interaction with $p\text{CO}_2$ ($F = 7.28$, $p < 0.05$). No significant correlation was found between GPP estimated by the two methods (GPP- ^{18}O and GPP- O_2 ; $r = 0.26$, $p > 0.05$, $n = 48$). The cumulative GPP- ^{18}O was 33.0 ± 3.4 and $29.0 \pm 2.3 \mu\text{mol O}_2 \text{ l}^{-1}$ for C and OA treatments, respectively. OW and G treatments presented higher cumulative values with 34.8 ± 2.3 and $38.5 \pm 2.4 \mu\text{mol O}_2 \text{ l}^{-1}$, respectively.

$\delta^{13}\text{C}$ -DIC in natural sample was $\sim 3\%$ (data not shown) and reached, as expected, $759 \pm 18\%$ after addition of labelled ^{13}C -sodium bicarbonate. ^{13}C -DIC did not significantly change during the course of the experiment and did not differ between treatments (Figure 6a, Table 2). The transfer from labelled DIC to POC was very rapid and efficient, allowing the detection of ^{13}C -POC enrichment on the first sampling day (d2; Figure 6b), and saturation was achieved already at d4. ^{13}C -POC enrichment did not differ between treatments (Table 2). $\Delta\delta^{13}\text{C}$ -POC increased to a final enrichment of $501 \pm 23\%$. NCP- ^{13}C was lower than NCP- O_2 exhibiting a decreasing trend (Table 3) and did not significantly differ between treatments (Table 2). Cumulative NCP- ^{13}C was 11.8 ± 0.6 and $11.4 \pm 0.2 \mu\text{mol C l}^{-1}$ for C and OA, respectively. The warmer treatments had slightly higher values of 12.3 ± 0.3 and $12.1 \pm 0.5 \mu\text{mol C l}^{-1}$ for OW and G, respectively.

Discussion

This experiment was designed to study the effects of ocean warming and acidification on the composition and functioning of an oligotrophic plankton community in the coastal NW Mediterranean Sea. The elevated temperature condition was very well controlled with an average offset between ambient and elevated temperature of $2.8 \pm 0.4^\circ\text{C}$. The current rate of warming in the coastal NW Mediterranean Sea has been estimated to range from 0.026 to $0.033^\circ\text{C yr}^{-1}$ (Bensoussan *et al.*, 2009), although temperature projections are difficult to obtain due to large regional differences. Given these rates, the average 2.8°C temperature increase as applied in our study was representative for the end of the century. Although $p\text{CO}_2$ was lower than targeted and therefore lower than commonly used in similar perturbation studies, high- $p\text{CO}_2$ values of ~ 610 – $690 \mu\text{atm}$ correspond to the level of atmospheric CO_2 projected for 2060 according to the RCP 8.5 scenario (Meinshausen *et al.*, 2011).

The *in situ* sampling was performed 1 day after the maximum surface chl *a* concentration was measured in the Bay of Villefranche (data not shown; but see <http://somlit-db.epoc.u-bordeaux1.fr/bdd.php>). Consequently, nutrients were depleted with levels very close to detection limits. NO_x was consumed rapidly in all treatments during the first 2 days of the experiment (from d0 to d2) and its

Table 2. Results of the permutational analysis of variance for selected parameters and processes.

	$p\text{CO}_2$		temperature		$p\text{CO}_2$: temperature	
	F	p	F	p	F	p
Carbonate chemistry						
Alkalinity	0.029	0.874	0.974	0.362	0.686	0.458
DIC	696.193	0.001*	0.171	0.685	0.656	0.443
$p\text{CO}_2$	555.319	0.001*	0.175	0.688	1.000	0.341
pH	678.988	0.001*	0.048	0.831	1.191	0.2917
Nutrients						
NO_x	4.017	0.078	0.551	0.482	0.792	0.387
PO_4^{3-}	13.187	0.005*	0.038	0.836	2.589	0.152
Silicate	0.969	0.376	0.350	0.587	1.652	0.222
Particulate organic matter						
POC	0.039	0.837	1.956	0.184	0.875	0.377
PON	0.000	0.990	0.135	0.729	3.240	0.093
POC : PON	0.050	0.855	0.030	0.876	3.713	0.079
Pigments						
Chl <i>a</i>	1.832	0.216	1.146	0.303	0.006	0.934
Fucoxanthin	1.254	0.285	0.548	0.479	0.004	0.966
19'-Hexafucoxanthin	0.384	0.537	2.570	0.125	0.210	0.656
Zeaxanthin	0.017	0.902	6.983	0.027*	0.037	0.842
Flow cytometry						
<i>Synechococcus</i>	0.332	0.566	6.106	0.028*	0.084	0.781
Pico-eukaryotes	8.694	0.016*	3.094	0.114	1.615	0.235
Nano-prokaryotes	0.057	0.816	0.093	0.788	0.042	0.837
Heterotrophic prokaryotes	0.145	0.729	1.392	0.268	0.002	0.974
Viruses	0.656	0.465	5.571	0.040*	0.291	0.588
^{13}C labelling						
^{13}C -DIC	1.746	0.204	0.214	0.642	4.592	0.057
^{13}C -POC	2.124	0.165	2.105	0.052	0.120	0.738
Metabolic rates						
GPP- ^{18}O	0.003	0.956	15.824	0.006*	7.283	0.021*
GPP- O_2	0.218	0.642	1.257	0.324	0.179	0.668
NCP- O_2	0.074	0.776	769.0	0.769	0.539	0.483
CR- O_2	0.139	0.728	1.683	0.240	0.035	0.849
NCP- ^{13}C	0.867	0.368	2.526	0.155	0.020	0.901

The "*" indicate significant effect ($p < 0.05$).

concentration significantly decreased while chl *a* concentration increased rapidly to a maximum on d2. This important chl *a* increase after enclosure, followed by decline owing to the depletion of inorganic nutrient, was also reported in other bottle experiments (De Madariaga and Fernandez, 1990; Scarratt et al., 2006) and can be due to a containment effect. In addition, it must be stressed that sieving onto 200 μm most likely limited the abundance of large zooplankton releasing predation pressure during the first days but favouring growth of micro-heterotrophs that in turn grazed on small phytoplankton. Although degradation pigments representative for grazer faecal pellets (phaeophytin *a* and phaeophorbid *a*) were close or even below detection limit throughout the experiment (data not shown), suggesting that there were no or very few zooplanktonic organisms, as the available seawater volume for sampling was not sufficient, zooplankton abundances were not estimated during this study. Based on pigment concentrations, prymnesiophytes were the dominant species and all phytoplankton groups decreased in abundance along the experiment, except for cyanobacteria. The dominance of small phytoplankton and the general decrease in biomass are consistent with post-bloom, nutrient-depleted conditions. Cyanobacteria and prymnesiophytes present a higher surface vs. volume ratio and have a higher affinity for nutrients than larger phytoplankton, cyanobacteria being even more competitive and able to grow under these very low nutrient conditions (e.g. Eppley

et al., 1969; Irwin et al., 2006). The dominance of pico- and nano-planktonic species at the end of the bloom period has already been observed in the Bay of Villefranche (Sheldon et al., 1992). While chl *a* concentrations showed very clear variations during the experiment, it was not the case for POC. These values presented large variations in between replicates and could explain the fact that we did not observe any temporal variation in POC.

The rates of community metabolism are comparable with those measured during a previous experiment performed in the bay of Villefranche in March 2003 (González et al., 2008). However, in contrast to this study, GPP- ^{18}O and GPP- O_2 were not correlated during our experiment. This can be explained by the relatively small range of values measured during our study in contrast to the large range of values (different seasons, depth, and sites) presented by González et al. (2008). Due to the limited amount of water available in the 4 l bottles, NCP- O_2 and CR- O_2 rates have been measured without replication. This led to relatively large uncertainties in the determinations of these rates and, even more important, in the estimates of GPP- O_2 . Although the same number of samples were available to estimate GPP- ^{18}O rates, the errors associated with this method were much smaller than with the O_2 light-dark techniques as GPP- O_2 is estimated based on two values (NCP- and CR- O_2) associated to large uncertainties. Therefore, the ^{18}O -labelling approach appears much more reliable for estimating GPP rates than the classical

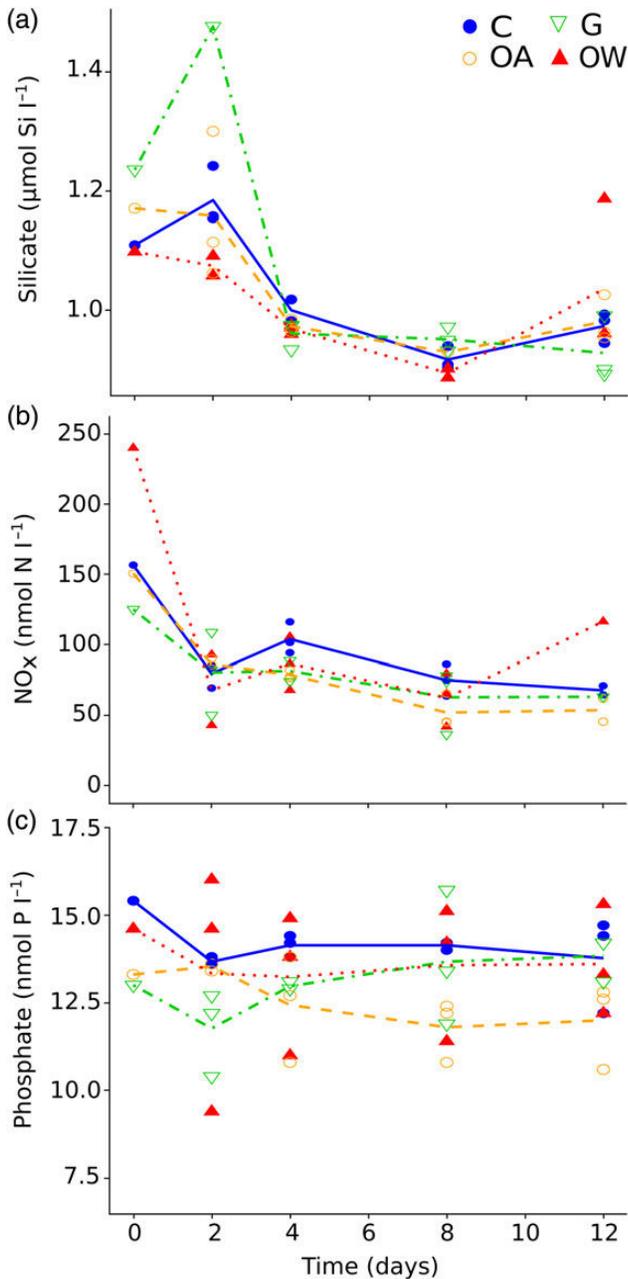


Figure 2. Concentration of inorganic nutrients as a function of time: (a) silicate, (b) NO_x ($\text{NO}_3^- + \text{NO}_2^-$), and (c) phosphate. Control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments. Symbols are for the three replicates of each treatment. Lines: solid (C), dashed (OA), dotted (OW), and dotted–dashed (G).

light–dark technique when rates are low and only a small sample volume is available. However, it must be stressed that the ^{18}O -labelling techniques does not allow estimating CR, which is critical for determining the autotrophic vs. heterotrophic behaviour of the community. Nevertheless, the observed decrease in $\text{GPP-}^{18}\text{O}$ during the experiment was consistent with the decrease in the phytoplankton biomass indicated by the pigments analyses.

The ^{13}C enrichment was successful and showed decreasing primary production rates along the experiment which is consistent with the observed decrease in phytoplankton biomass and GPP

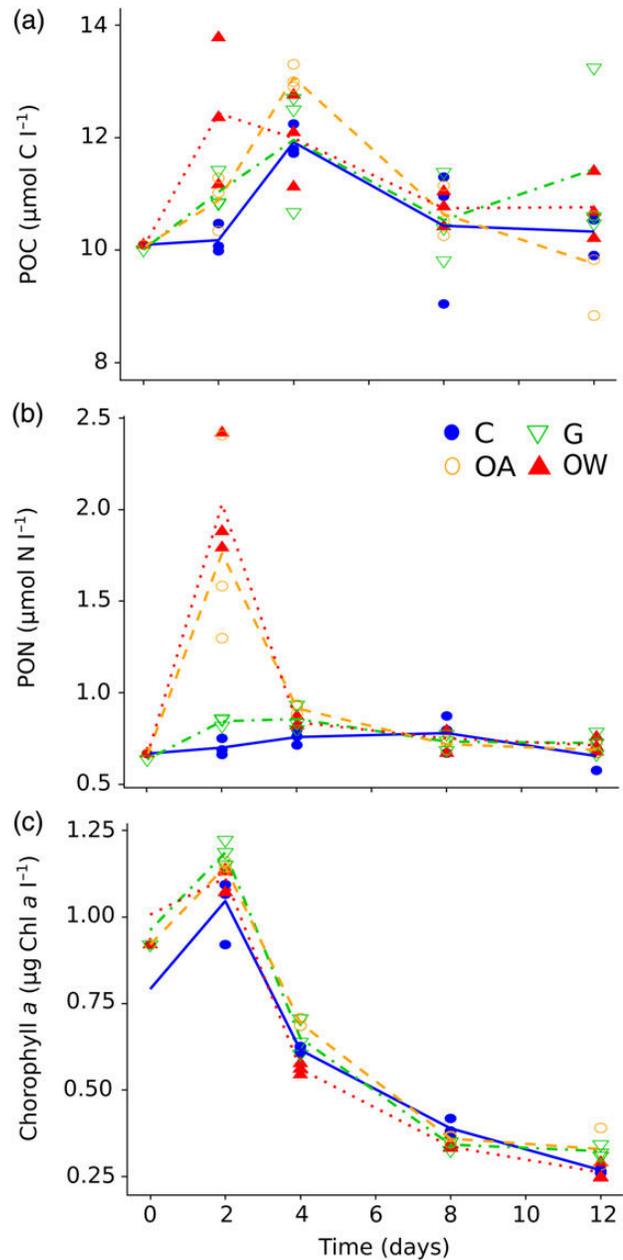


Figure 3. Particulate organic matter and chl *a* as a function of time: (a) POC, (b) particulate organic nitrogen (PON), and (c) chl *a* measured by HPLC. Control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments. Symbols are for the three replicates of each treatment. Lines: solid (C), dashed (OA), dotted (OW), and dotted–dashed (G).

measured by the ^{18}O method. However, the NCP estimated based on the ^{13}C method ($\text{NCP-}^{13}\text{C}$), representing the freshly labelled material produced by phytoplankton, was lower than NCP estimated by the light–dark method (NCP-O_2). The $\text{NCP-}^{13}\text{C}$ and NCP-O_2 were measured from incubation in different volumes (60 ml vs. 4 l) and over different periods (24 h vs. 2 or more days), which might explain part of this difference. Moreover, it is likely that, a significant part of the organic material produced was released in the DOC pool (Wood and Van Valen, 1990; Lopez-Sandoval *et al.*, 2011) which was not measured during our experiment.

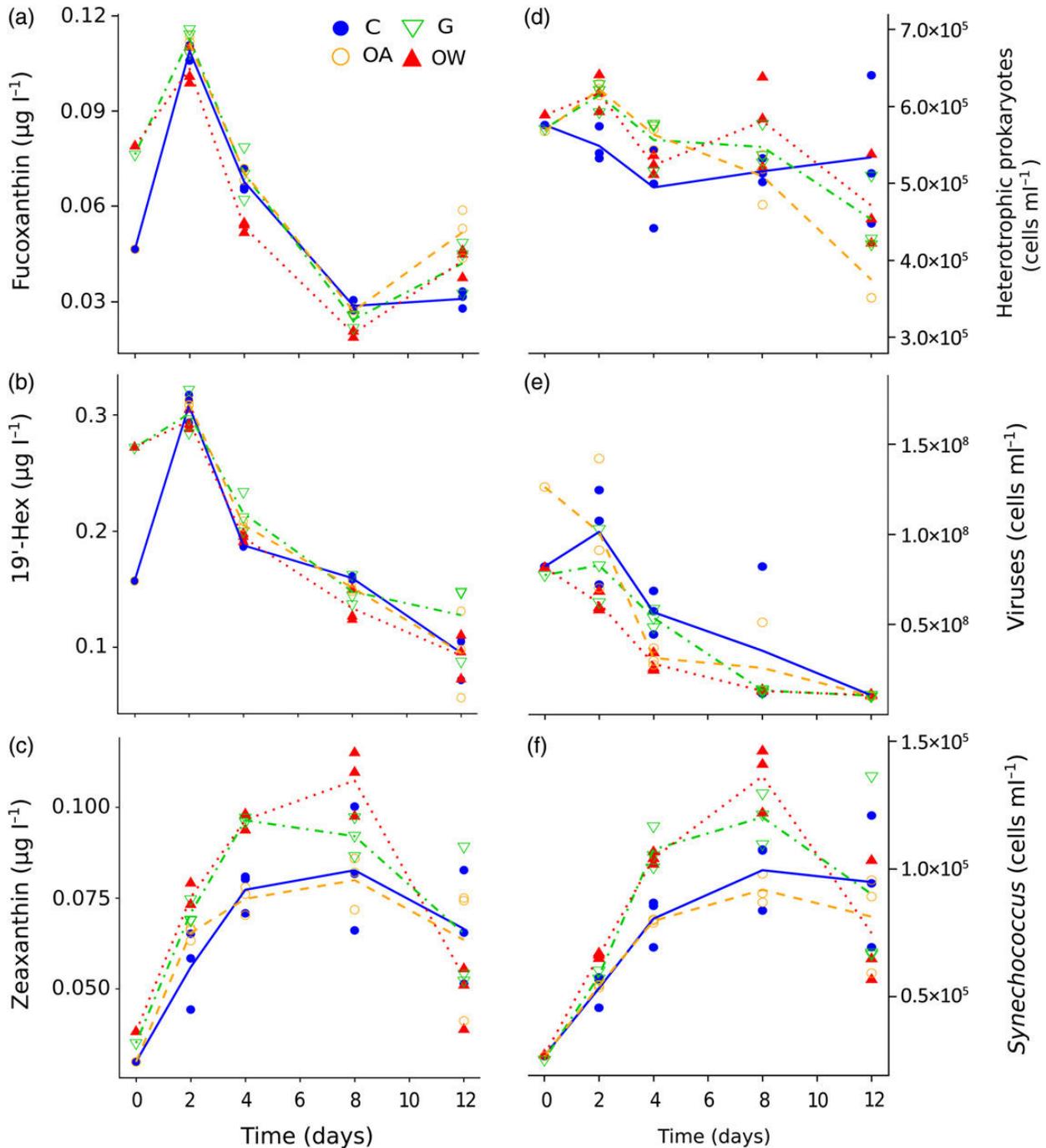


Figure 4. Pigments (left panels) and flow cytometer counts (right panels) as a function of time: (a) fucoxanthin (typically diatoms), (b) 19'-hexanoyloxyfucoxanthin (19'-hex; prymnesiophytes), (c) zeaxanthin (cyanobacteria), (d) abundance of heterotrophic prokaryotes, (e) abundance of viruses, and (f) abundance of *Synechococcus*. Control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments. Symbols are for the three replicates of each treatment. Lines: solid (C), dashed (OA), dotted (OW), and dotted-dashed (G).

During the recent years, an experimental effort to study ocean warming and acidification effects at the plankton community level has been initiated; however, there is still a clear lack of information for low productive oceanic regions (oligotrophic) that represent an important, and expanding surface of the global ocean (e.g. Irwin and Oliver, 2009), having then a significant role in carbon cycling. The present experiment was designed to investigate the effect of both ocean acidification and warming under conditions that prevail for

most of the year in the oligotrophic Mediterranean Sea, i.e. very low nutrient availability and a community dominated by small phytoplankton species.

In our study, no significant effects of elevated temperature and/or CO_2 were found for most parameters and processes (Table 2). As reported in other experiments (e.g. Feng et al., 2009), C:N ratio was not affected either by temperature or CO_2 . The $p\text{CO}_2$ effect detected on the phosphorus concentration is most likely due to sampling

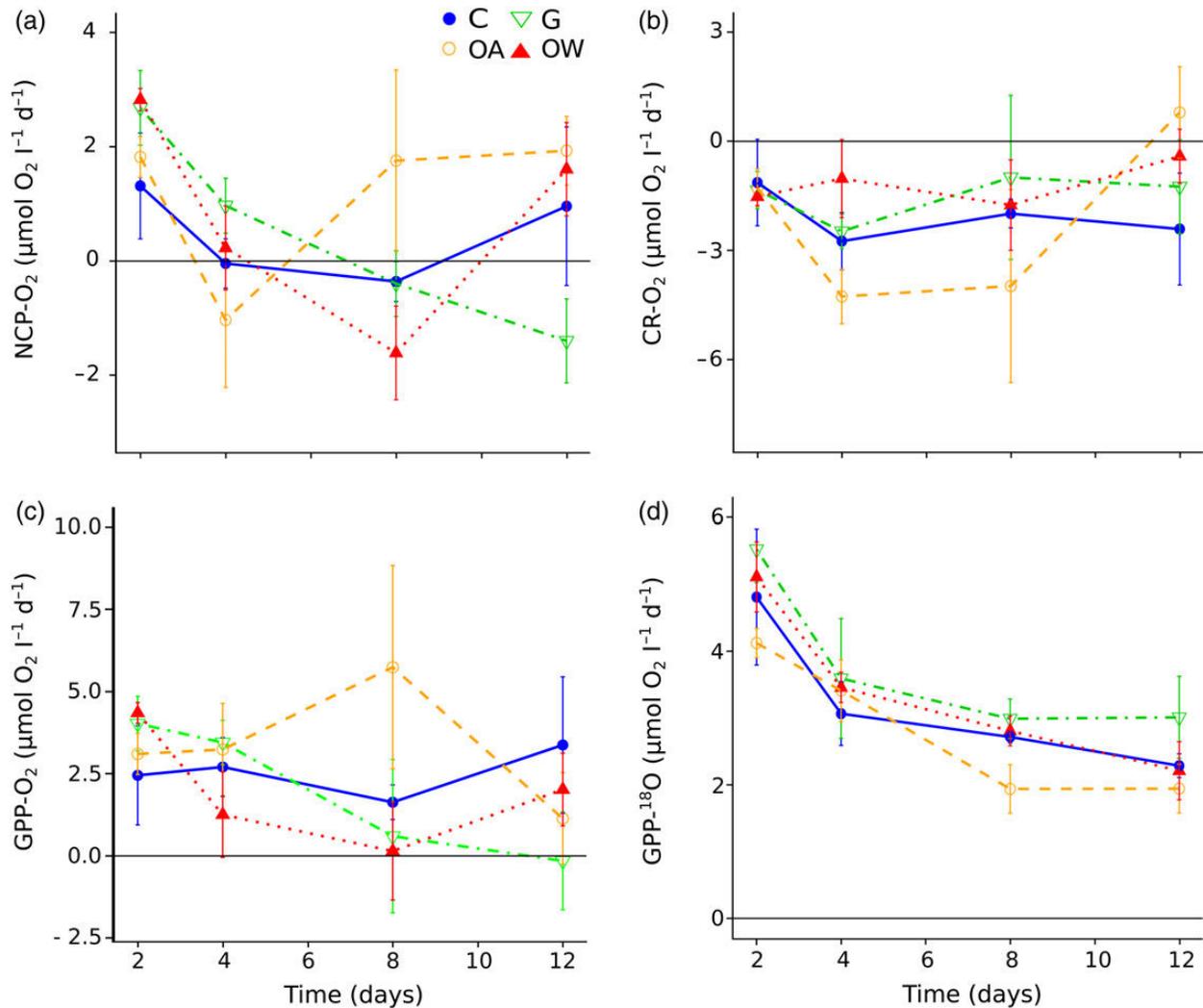


Figure 5. Community metabolism as a function of time: (a) NCP (NCP-O₂), (b) CR (CR-O₂), (c) gross primary production using the O₂ techniques (GPP-O₂), and (d) gross primary production using the ¹⁸O-labelling technique (GPP-¹⁸O). Control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments. Symbols are for the three replicates of each treatment. Lines: solid (C), dashed (OA), dotted (OW), and dotted-dashed (G).

and/or analytical uncertainty. Indeed, during the whole experiment, concentrations varied within a very small range of ± 5 nmol l⁻¹ and for most of the sampling days, differences between replicates were larger than between treatments. The GPP-¹⁸O rates were higher in warmer treatment and even more enhanced when combined with high CO₂ conditions. This is in agreement with observed stimulations of the metabolism by temperature (Eppley, 1972; Toseland *et al.*, 2013). Our data also support the previous findings of studies with higher nutrient levels in which elevated pCO₂ exerts a moderate effect on primary production rates unless it is combined with an increase in temperature (bottles incubation <3 l; Hare *et al.*, 2007; Feng *et al.*, 2009). An experiment performed in larger volumes (3000 l) has shown an increase in DOC production as well as photosynthetic activity and decrease in POC production in warmer and high CO₂ level conditions while gross community production remained unchanged (Kim *et al.*, 2011, 2013).

It must be mentioned that although no study combining both drivers were conducted in oligotrophic conditions, ocean acidification alone has been reported to decrease DOC production in the

nutrient-depleted Okhotsk Sea at high CO₂ level while POC production was unchanged (Yoshimura *et al.*, 2010). Furthermore, in iron-limited areas (Bering Sea and North Pacific) presenting similar chl *a* level than in our study, effects of ocean acidification were investigated and have shown different effect on POC and DOC accumulation that have been related to differences in community structure (Yoshimura *et al.*, 2013). Indeed at the site where small phytoplankton species (70% picoeukaryotes and 20% *Synechococcus*) were dominant, no effect was detected, as in our study, while at the site where diatoms were dominating (75%), POC accumulation was smaller at high CO₂ levels. In our study, cyanobacteria (comprising *Synechococcus* and *Prochlorococcus* spp.) is the only taxonomic group that has shown enhanced abundance in warmer conditions though to a lesser extent when combined with high CO₂ level. Indeed, while the increasing tendency in both warming and greenhouse treatments was similar during the first sampling days, elevated pCO₂ appeared as unfavourable to this population after day 8 of our experiment. This is partially consistent with single-cell experiments that have shown no effect of increased CO₂ alone on *Synechococcus*

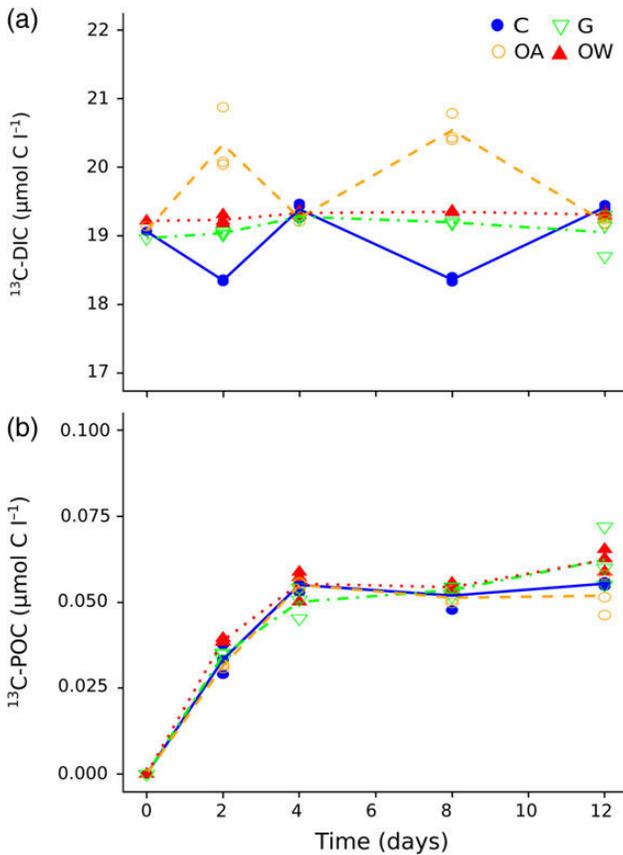


Figure 6. Concentrations of labelled (a) dissolved inorganic carbon (DIC) and (b) POC expressed in $\mu\text{mol C l}^{-1}$. Control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments. Symbols are for the three replicates of each treatment. Lines: solid (C), dashed (OA), dotted (OW), and dotted–dashed (G).

Table 3. NCP based on ^{13}C enrichment in the control (C), ocean warming (OW), ocean acidification (OA), and greenhouse (G) treatments (average \pm SD).

Day	NCP- ^{13}C ($\mu\text{mol C l}^{-1} \text{d}^{-1}$)			
	C	OW	OA	G
2	1.93 ± 0.26	2.14 ± 0.05	1.69 ± 0.06	2.00 ± 0.06
4	1.52 ± 0.05	1.54 ± 0.13	1.60 ± 0.03	1.46 ± 0.13
8	0.76 ± 0.05	0.76 ± 0.02	0.71 ± 0.03	0.79 ± 0.02
12	0.52 ± 0.01	0.58 ± 0.03	0.51 ± 0.06	0.62 ± 0.08

growth rates and significant effects of temperature alone or in combination with acidification (greenhouse; Fu *et al.*, 2007). However, in contrast to our results, growth rates were not significantly different between the elevated temperature and the greenhouse treatments. Furthermore, the same study reported no effect on *Prochlorococcus*, while increased abundances in the warmer treatment were observed in our study (data not shown). Finally, in a natural community of *Synechococcus* and *Prochlorococcus* spp. carbon fixation rates were also not affected by acidification under both nutrient replete and P-limited conditions (Lomas *et al.*, 2012).

Extrapolating our findings to *in situ* oceanic conditions and facilitating potential future comparison studies necessitates a

discussion on the limits of the experimental design considered in the present study. Indeed, it must be stressed that our experiment was performed in relatively small-enclosed bottles (4 l) that undoubtedly led to some confinement effects which constrained the plankton community response (i.e. no turbulence, no nutrient re-supply, no movement through the euphotic zone, etc.; Scarratt *et al.*, 2006). In our study, cyanobacteria species observed during the incubations were not N_2 fixers; however, it must be stressed that some strains have been shown to increase their N_2 fixation rates under high CO_2 conditions (e.g. Hutchins *et al.*, 2009). The presence of N_2 fixers species in the community could have had influenced the results reported here. In addition, seawater was sieved through $200 \mu\text{m}$ to remove large organisms meaning that we did not take into account the complete community and therefore these findings are valid only when top-down control is negligible (Kim *et al.*, 2013). Any potential change in the top-down pressure, due to change in macrozooplankton enhanced grazing in warmer and/or acidified condition, was therefore not investigated in this study. To assess the potential effect of climate change on communities of two or more trophic levels and trophic interactions (e.g. from phytoplankton to macrozooplankton), large mesocosm ($\sim 50 \text{m}^3$) studies are more appropriate as whole communities can be trapped and *in situ* temperature, irradiance and water masses are maintained close to “real world” conditions (Riebesell *et al.*, 2010, 2013).

It must be stressed that a rise in primary production with elevated temperature is not foreseen on a global scale as many studies report a decrease of primary production as a consequence of a stronger stratification of the water column which limits nutrient supply to the surface mixed layer (Bopp *et al.*, 2005). The observed increase in abundance of cyanobacteria in the warmer treatments in our study is in accordance with *in situ* observations, as it is recognized that these species have a wider temperature range than other phytoplankton species. Cyanobacteria will probably benefit from climate change as long as the temperature does not exceed their thermal tolerance (Morán *et al.*, 2010; O’Neil *et al.*, 2012). A potential shift toward small species could lead to diminished energy transfer to microzooplankton as their lipid content is much lower than it is for larger phytoplankton species (e.g. Von Elert and Wolffrom, 2001). Furthermore, the carbon export efficiency depends on the community structure and a shift to smaller species will probably lead to a less efficient carbon export under elevated temperature (Bopp *et al.*, 2005). As nutrients (N and P) were highly limiting, the absence of effect by ocean acidification alone does not appear as a surprise. Unfortunately, no data on dissolved organic nutrient concentrations are available and we could not precisely investigate any modifications in nutrient acquisition mechanisms (inorganic vs. organic) under the different treatments. Nevertheless, as no change of plankton community composition and functioning were shown at elevated $p\text{CO}_2$, it appears that the community did not take advantage of the increased CO_2 availability. To conclude, this study on the combined effect of both drivers has shown that ocean acidification and warming in isolation do not have the same effect on cells abundances and production rates than when combined, emphasizing the need to study these two drivers synergistically.

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Supplementary Material



Supplementary Material: Figure SM-IV-1. Picture of the experiment showing the two outdoor tanks on the pontoon in Villefranche-sur Mer (left) and the + 3°C with the 4 L bottles, the heater and pump for water circulation (right).

Chapter V

Synthesis and general discussion

This chapter aims at synthesising the results presented in the previous chapters and to discuss them in a more general context through an extensive literature review.

Plankton community in a high $p\text{CO}_2$ LNLC area

The aim of this work was to provide data on plankton community response to two anthropogenic drivers, ocean warming and acidification in ecosystems that have been chronically under studied although they represent a large portion of the ocean surface area and global primary production: oligotrophic regions. Past experiments have been mainly focused on eutrophic or mesotrophic systems (e.g. Tortell et al., 2002) or conducted with nutrient enrichment (e.g. Riebesell et al., 2007) and provided contradictory results on the effect of these stressors on the structure and functioning of plankton communities, leading to the absence of a general consensus. Furthermore, very few experiments combining ocean acidification and warming have been performed.

The Mediterranean Sea was selected as a study area with low nutrient low chlorophyll (LNLC) levels in the frame of the European MedSeA project. The potential effects of ocean warming and/or acidification in this region were assessed following three null hypotheses:

1. Ocean acidification will affect phytoplankton primary production

During the two mesocosms experiments that have been conducted in the Northwestern Mediterranean Sea, the response of plankton metabolic rates to six levels of CO_2 following a gradient approach was assessed with different methods to overcome the limitations inherent to each technique. In addition, another experiment performed in smaller volumes was conducted to test for the effect of an increase in $p\text{CO}_2$ to a value of $\sim 700 \mu\text{atm}$, in combination or not with an increase in temperature ($\sim 3 \text{ }^\circ\text{C}$). These three experiments were conducted at different periods: during the summer-stratified period and under pre-bloom conditions (winter/spring) for the mesocosm experiments and after the bloom for the experiment performed in small volumes. For all these experiments, seawater had low concentrations of nutrients and chlorophyll levels and no nutrient was added. Measured plankton metabolic rates were within the range of rates previously reported for this area at the periods considered. The results of these experiments have shown no effect of increased $p\text{CO}_2$ alone on particulate and dissolved primary production nor on community respiration. These results obtained in nutrient-depleted conditions are not supporting the hypothesis that increasing CO_2 levels will enhance primary production by lowering the CO_2 acquisition cost for phytoplankton species (e.g. Riebesell, 2004).

2. Some species will be more affected than others by ocean acidification

It is suspected that some phytoplankton groups will be favoured compared to others due to differences in the efficiency of their carbon concentrating mechanisms (CCMs; e.g. Reinfelder, 2011). During the two mesocosm experiments, ^{13}C enrichment was performed in order to trace and quantify carbon flows within the plankton community. This methodological approach, coupled to isotopic analysis of biomarkers (phospholipid fatty acid; PLFA) on plankton community has, to the best of our knowledge, never been used in relatively unproductive plankton communities such as those found in most of the Mediterranean Sea. This approach has successfully provided insights on carbon fixation rates by different phytoplankton groups and confirmed the absence of a CO_2 effect on these communities, in agreement with results obtained with other methods (see previous hypothesis). However, determination of PLFA has proven to be difficult under the tested conditions because concentrations were very low and their profiles in Mediterranean species are unknown. Further developments of this interesting technique are necessary to improve its reliability in oligotrophic seas. This will be further discussed in the “Conclusion and perspectives” section.

3. Under multiple-stressors such as ocean acidification and warming, the effect of one driver dominates

Ocean acidification and ocean warming will occur concomitantly and both drivers have potential effects on plankton community. Their combined effects were investigated by means of a bottle experiment that was conducted in the Bay of Villefranche. While the abundance of most phytoplankton species, bacteria and viruses communities collapsed over the 12 days of the experiment, the abundance of cyanobacteria (mostly *Synechococcus* and *Prochlorococcus*) increased with enhanced abundance in the warmer treatment. In the treatment combining ocean acidification and warming, higher CO_2 levels tended to limit this increase. This could have consequences on carbon export to deeper layers (e.g. Bopp et al., 2005) and to higher trophic levels as cyanobacteria populations have lower nutritive value than other phytoplankton species (e.g. Von Elert and Wollfrom, 2001). Gross primary production rates were also enhanced in warmer conditions. These results, suggest that the Mediterranean plankton community response is mainly driven by temperature increase rather than by acidification despite some interactions between drivers, emphasizing the necessity of performing multi-drivers experiments.

The present work has brought some clues to the questions raised by the scientific community during the last decades about the effect of climate change on plankton communities. All together, the experiments performed in this work have provided coherent results and all showed that ocean acidification will likely have limited impacts on plankton communities of the NW Mediterranean Sea and that ocean warming could favour the abundance and production of small species such as cyanobacteria that have a low capacity for carbon export. Under low concentration of nutrients and chlorophyll *a* levels, the metabolic rates measured were unchanged by the increase in CO₂. This does not seem very surprising as, nitrogen and phosphorus were more limiting than CO₂ that was abundant enough to support the phytoplankton CO₂ demand. As a consequence of low nutrient availability, metabolic rates were not affected by the increase in CO₂ availability, as no other resources were available for photosynthesis. Increasing seawater temperature by 3 °C relative to control did however increase metabolic rates despite the low nutrient concentration.

The response of plankton community to climate change seems to be mostly driven by environmental parameters other than those of the carbonate system (i.e. nutrient availability, light, temperature, etc). These parameters, that determine plankton community temporal dynamics and structure, are very variable in space and time. Therefore, the effect observed in the NW Mediterranean Sea might not be found in other low nutrient systems, this will be further discussed in the next section.

Ocean acidification effect on plankton community in other ecosystems

In the discussion section of the previous chapters, our results were compared with those of similar experiments. Here we propose a comprehensive review of the ocean acidification effect on plankton metabolism and biomass based on an extensive literature survey. For about 20 years now, experiments on ocean acidification have been performed and there is a need for the scientific community to clarify the recent knowledge and try to find a consensus to communicate with more certainties on the biological response to this driver. Indeed, after several years of experiments on the effect of ocean acidification on plankton community it seems that its response depends on the region, plankton community composition and the nutrient availability. Since the last review of Riebesell and Tortell (2011), several experiments looking specifically at ocean acidification effect at plankton community level have been performed in different locations.

Twenty experiments have been reported at different locations (Table V-1) and data on biomass and primary production have been collected through 23 different publications (from 1997 to 2014). Half of these publications have been published in the last 3 years. Details on chlorophyll *a* levels (as a proxy for biomass) and primary production rates measured in these experiments are available in Appendix B. In order to provide quantitative data, the response ratio for chlorophyll *a* (RR-chl *a*) and primary production (RR-PP) were calculated as the ratio of the effect at elevated CO₂ level relative to control conditions at each time step. As in several experiments only final biomass and production values were available, thus only final response ratios were considered for all studies, except for the Svalbard experiment for which final response ratios at the end of each experimental phase were considered. Depending on the experiment, different values of CO₂ levels have been tested in areas with different initial *p*CO₂ conditions. Therefore, we selected only studies with elevated CO₂ levels lower than 4x initial control *p*CO₂ levels. Finally, when an effect was reported at several CO₂ levels, response ratios were calculated for each level. When no effect was detected despite different tested CO₂ levels it was reported as a single value of 1. For most of the experiments, data were available in the papers and/or in the ocean acidification database hosted in the World Data Centre Pangaea (<http://www.pangaea.de>). However, the nutrient and chlorophyll *a* data from Hein and Sand-Jensen (1997) and Riebesell et al. (2000) were not available. As these experiments have been performed in locations of frequently sampled sites, initial chlorophyll *a* concentrations were taken from the AMT cruise reports (http://www.bodc.ac.uk/projects/uk/amt/cruise_programme/) and Wong et al. (2005), respectively.

Table V-1. List of experiments considered in our literature survey.

Location	Date	Reference	Number of related pub.	Number of studies	Additional information
S Atlantic	-	Hein and Sand-Jensen (1997)	1	18	Few environmental parameters details
N Pacific	1998-1999	Riebesell et al. (2000)	1	5	Few environmental parameters details
Bergen, Norway (PeECE I)	2001	see Riebesell et al. (2008)	2	1	Nutrient addition
Bergen, Norway (PeECE II)	2003	see Riebesell et al. (2008)	1	1	Nutrient addition
Bergen, Norway (PeECE III)	2005	see Riebesell et al. (2008)	3	1	Nutrient addition
Oshtock Sea, NE Pacific	2006	Yoshimura et al. (2010)	1	1	
Ross Sea, Antarctica	2006	Tortell et al. (2008)	1	1	
Denmark	2007	Nielsen et al. (2010)	1	2	
Bering Sea and N Pacific	2007	Yoshimura et al. (2013)	1	2	
Tasmania	2007	Nielsen et al. (2012)	1	1	
N Pacific	2008	Endo et al. (2013)	1	2	Fe enrichment
Japan	2009	Hama et al. (2011)	1	1	Nutrient addition
Godvari estuary, India	2009	Biswas et al. (2011)	1	2	
Bering Sea	2009	Sugie et al. (2013)	1	2	Fe enrichment
BATS station, Atlantic	2009-2010	Lomas et al. (2012)	1	3	Cyanobacteria population
Svarlbard	2010	see Riebesell et al. (2013)	4	1	Nutrient addition
Weddel sea, Antarctica	2010	Hoppe et al. (2013)	1	2	Fe enrichment
NW European continental shelf	2011	Richier et al. (2014)	1	5	
Bay of Calvi, Corsica	2012	this thesis	1	1	
Bay of Villefranche, France	2013	this thesis	1	1	

The entire database has been first explored by counting the number of studies. It is considered as a study here: 1) different geographical sites (e.g. Yoshimura et al., 2013 have performed two studies) except for Hein and Sand-Jensen (1997) who did 18 stations and Riebesell et al. (2000; 5 studies) as there are no available information on the different sampling stations, 2) results from different experimental conditions tested in one site (e.g. iron limited or replete conditions; Richier et al. (2014) have performed 5 studies), 3) when different measurement methods are available for one experiment they have been considered as different studies (e.g. for PeECE III in 2005 there are three studies for metabolic rates) and 4) specifically to the Svalbard mesocosm experiment the three temporal phases have been considered as different studies.

In total 31 and 38 studies for RR-chl *a* and RR-PP respectively, have been taken into account. Most (~ 60%) of the studies reported no effect of ocean acidification on chlorophyll *a* and/or primary production (Figure V-1) while ~ 22% reported a positive effect and 16% of the studies have shown a negative effect.

The first hypothesis that can be tested, based on this dataset, is whether the lack of ocean acidification effect on plankton biomass and primary production is more prevalent under low nutrient concentration. In oligotrophic areas, plankton communities being nutrient-limited, an increase in CO₂ will lead to an increase in primary production and biomass. This is not validated here as the response ratio of chlorophyll *a* and primary production response ratios did not show any clear pattern when related to dissolved inorganic nitrogen (NO_x as nitrate and nitrite; Model II linear regression; RR-chl *a*: n = 52, r² = 0.06, *p* > 0.05; RR-PP: n = 71, r² < 0.01, *p* > 0.05) and phosphorus (DIP; RR-chl *a*: n = 52, r² = 0.01, *p* > 0.05; RR-PP: n = 70, r² < 0.01, *p* > 0.05) concentrations or NO_x/DIP ratio (RR-chl *a*: n = 52, r² = 0.06, *p* > 0.05; RR-PP: n = 70, r² < 0.01, *p* > 0.05).

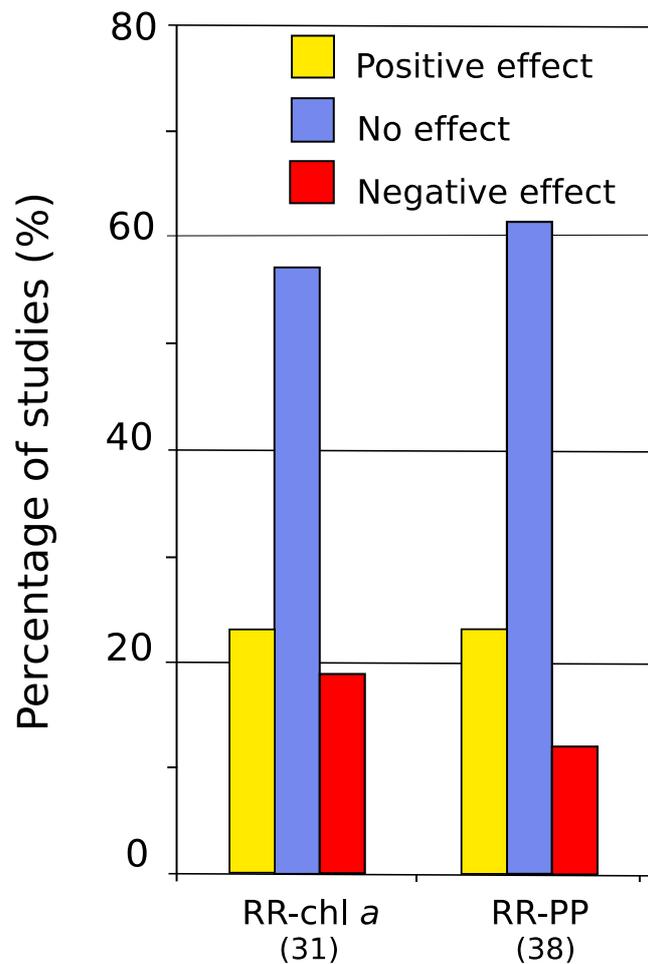


Figure V-1. Number of studies expressed as a percentage showing a positive effect (stimulation; yellow bars), no effect (blue bars) and negative effect (inhibition; red bars) for all the studies considered. RR-chl *a* and RR-PP stand for response ratio of chlorophyll *a* and primary production respectively and the number of studies are indicated in parenthesis.

The second hypothesis that can be tested is whether the initial chlorophyll *a* concentrations determines the response of a community to increased $p\text{CO}_2$ (Figure V-2). A low initial chlorophyll *a* biomass might be related to relatively unproductive ecosystems due to environmental limitations (such as nutrient but also irradiance, temperature, etc...) thus an increase in CO_2 will have no effects. This hypothesis is partly supported by the data as shown with Model II linear regressions using the logarithm of initial chlorophyll *a* concentrations, although only for biomass (RR-chl *a*: $n = 52$, $r^2 = 0.18$, $p < 0.05$), while for primary production, no significant relationship was found (RR-PP: $n = 70$, $r^2 = 0.03$, $p > 0.05$). Keeping in mind that most experiments did not show any CO_2 effect, studies with initial chl *a*

$\geq 0.5 \mu\text{g L}^{-1}$ present the tendency to be more often positively affected by increased CO_2 , with some exceptions.

A third hypothesis to test is whether community composition rather than biomass and/or nutrient availability determines the response to ocean acidification. Indeed, phytoplankton species have different CO_2 supply requirements and have adapted to some extent to marine environmental constraints through evolution (e.g. CCM). Studies for which RR-chl $a < 1$ presented community compositions dominated by diatoms and dinoflagellates, communities with RR-chl $a = 1$ were almost exclusively composed of cyanobacteria (mainly *Synechococcus*) and small haptophytes and studies with RR-chl $a > 1$ were a mix of studies with dinoflagellates, cryptophytes as well as small haptophytes and cyanobacteria. For RR-PP, no general trend is found as communities dominated by diatoms, dinoflagellates, cyanobacteria or haptophytes presented RR-PP < 1 . It must be noticed that papers almost never provide a quantitative method to describe community structure. Different analytical methods were used to quantify diversity and abundance such as flow cytometry, microscopy cell counts and/or pigment analyses and therefore some species could be present but not measured. For example, *Synechococcus* and *Prochlorococcus* are very well quantified by flow cytometry but not all studies use this method. In the early days of ocean acidification research, several experiments performed on single species in culture have shown that increased CO_2 enhances photosynthesis, carbon fixation, growth rates and elemental composition (see Riebesell and Tortell, 2011 for a review). Single strain cultures of cyanobacteria have, for example, shown increase in photosynthesis, cell division and elemental composition in response to ocean acidification (see Riebesell and Tortell, 2011 for review; Fu et al., 2007). However, this is not supported by experiments conducted on communities dominated by this group, in which no effect (this thesis; Yoshimura et al., 2010; 2013; Lomas et al., 2012) or even a negative effect (Paulino et al., 2008; post-bloom conditions) have been observed. There is the exception of Endo et al. (2014) who reported an increased cyanobacteria biomass in the North Pacific under increased $p\text{CO}_2$. It seems that only looking at the community composition and physiological characteristics of the species present is not sufficient to explain the different biological responses observed. The sum of the species-specific responses does not reflect the response of the natural assemblages. We suggest that it is more likely that the initial ratio of the different phytoplankton groups will explain the differences. Indeed, as it has been recently demonstrated, the initial ratio between cyanobacteria, diatoms and dinoflagellates has more effect on biomass dynamics than ocean

acidification (Eggers et al., 2014). Small differences in species composition can then cause large differences in ecosystem functioning (Eggers et al., 2014) and could partly explain the large variability of responses observed for primary production rates (RR-PP). However, this does not explain why similar communities with similar relative species composition are positively or negatively affected by ocean acidification.

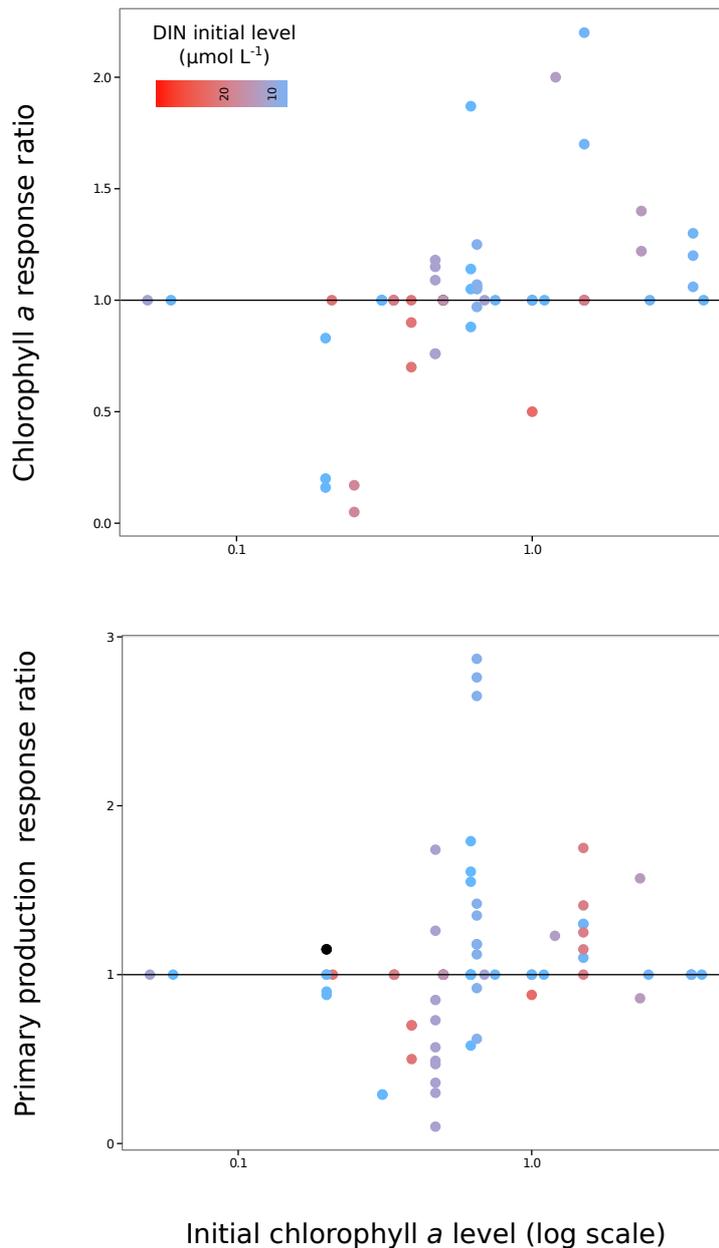


Figure V-2: Response ratio for chlorophyll *a* (upper panel) and primary production (lower panel) relative to initial chlorophyll *a* concentration ($\mu\text{g L}^{-1}$; log scale). The colour gradient corresponds to initial nitrogen (NO_x) concentration ($\mu\text{mol L}^{-1}$), black is used when no data were available.

A fourth hypothesis is that the type of trophic pathway dominating the ecosystem at the period at which the experiment is performed is important as well as the experimental set-up. Throughout the year the dominant trophic pathway, i.e. classic vs. microbial food web, shifts along a continuum based on nutrient availability, hydrological conditions and predation pressure by zooplankton. Trophic interactions in the community could therefore dampen ocean acidification effects (Rossoll et al., 2013) through changes in the grazing pressure on the different phytoplankton groups. This hypothesis is very difficult to test as many studies do not report on grazing rates or secondary producers composition. Furthermore, it must be stressed that most experiments do not consider the complete community as seawater is usually sieved to remove large organisms (e.g. in our experiments).

The initial hypothesis that, plankton communities are not influenced by increased CO₂ has not been verified based on this literature review and a conceptualization of ocean acidification effects based on nutrient levels cannot be proposed. The same is true for initial community composition that did not allow to fully explain the differences observed between experiments. Primary production is a key parameter in climate change research as it partly determines the ability of an ecosystem to store or release CO₂ in/from the ocean. The response of primary production to acidification is more complex to conceptualize than biomass. As suggested by Hein and Sand-Jensen (1997), the relative abundance of species in the natural assemblages will be more likely impacted than primary production. As discussed previously, most studies performed at community level have shown unchanged primary production rates as obtained by several incubation and analytical methods. This neutral effect would have profound consequences, as in contrast to what had been initially suggested (e.g. Riebesell et al., 2007), the biological pump will not increase as a consequence of ocean acidification and thus will not help sequestering anthropogenic carbon in the ocean while the solubility pump will decrease as a consequence of warming.

In the literature review, experiments combining both ocean acidification and warming have not been selected. Although very few experiments have looked at the combined effect of these drivers (Feng et al., 2009; Hare et al., 2007; Kim et al., 2008; 2011; Kim et al., 2013), they converged to the conclusion that ocean warming will lead to enhanced primary production rates with little or no interaction with ocean acidification. Our findings support the fact that ocean warming could have more effect than ocean acidification on plankton communities structure and functioning.

The experimental approaches used in this thesis as well as in past experiments are associated to some common limitations. These experiments do not allow investigating the long-term effect of ocean acidification and warming under close to *in situ* conditions while following seasonal variability. It can be expected that plankton communities evolves to adapt to future high CO₂ conditions and take advantages of these environmental modifications. Furthermore, even though this work has partly considered two drivers, others stressors exist in the natural environment and have not been taken into consideration in the present study. **These two limitations are related to the complexity of marine ecosystems and it is of the utmost importance for future research to take into account these limitations in the experimental design and data reporting in order to refine our projections of plankton structure and functioning in a high CO₂ ocean.**

Thus, based on these experimental findings, we are still unable to derive a consensus view of the response of biomass and primary production response to ocean acidification while some models have proposed a conceptualization of phytoplankton biomass and C:N stoichiometry responses to increased CO₂ levels (Verspagen et al., 2014). In agreement with our results, Verspagen et al. (2014) suggested that phytoplankton biomass is not likely influenced by ocean acidification in oligotrophic waters although the phytoplankton carbon:nutrient ratio would likely increase. Although models are useful tools for projecting future effects of anthropogenic stressors, an over-simplification of the models certainly leads to ignore most of the complexity of marine ecosystems. For example, Flynn et al. (2012) projected that small species will be more impacted by ocean acidification than large cells as they are less used to large pH changes in their immediate surroundings. This is definitely not supported by our results nor by the literature review that we performed, potentially because this model involves too many inadequate assumptions such as the one considering that all phytoplankton cells as spherical and non-motile objects with neutral buoyancy, obviously very unlikely to occur in nature.

Experiments at the community level, are more appropriate to take into consideration these natural aspects although no experimental design is exempt of bias. Within the experimental approaches and designs available, the use of large mesocosm facilities allows being as close as possible to “real-world” conditions. Over the last 30 years, mesocosms (here defined as large volumes > 1 m³) have been increasingly used (see Riebesell et al., 2010 for review) to gather data on the response of plankton communities to climate change (see

Stewart et al., 2013 for a comprehensive review). Natural environmental conditions such as light, seawater movement, and temperature changes are well reproduced although caution must be taken before making generalisation (Stewart et al., 2013). In large mesocosm systems, two or more trophic levels can be investigated simultaneously but the numbers and types of drivers that can be tested are limited for technical reasons (i.e. cost, logistics). To overcome this limitation, some experiments have been performed using semi-continuous cultures (e. g. Hutchins et al., 2003) but this requires installation and the algae are maintained under a constant physiological state (e.g. growth phase or stationary phase) that does not reflect *in situ* conditions as plankton's physiology naturally varies following variations in environmental conditions. Batch cultures (simple closed bottles) have also been used and are easier to set-up. Despite the inconvenience of the bottle effect, experiments in small volumes allow replication and a good control on the perturbed parameter, which is not always possible in larger volumes. The two different approaches (large mesocosm and small bottles) appear as complementary tools to investigate the effects of climate change on plankton community. **One of the directions highlighted the last years for future experiments is the complementary use of perturbation experiments (such as mesocosm), field observations (using natural gradients and time series for example) and modelling activities. This will require strong collaboration between experimentalists and modellers as well as interdisciplinary and coordination in the scientific community.**

Conclusions and perspectives

The three experiments that were conducted at different sites and periods during this thesis have shown coherent results and suggest that ocean acidification alone will not lead to significant modifications of plankton metabolic rates (i.e. net community production and respiration and gross primary production) as measured by different methods in the NW Mediterranean Sea. However, combined effects between ocean warming and acidification have been found. Ocean warming have enhanced gross primary production and shifted plankton community structure toward species that are less efficient for carbon export to deep-sea and higher trophic level.

Although metabolic processes as measured by different methods did not always correlate, due to differences in terms of incubation volume and time, they were within the range of rates reported in similar areas of the Mediterranean Sea. In addition, a technique to estimate group-specific production rates and carbon flow within the community based on carbon 13 labelling combined with the analysis of biomarkers (polar lipids fatty acids, PLFA) has been successfully applied for the first time in the frame of a mesocosm experiment in the Mediterranean Sea. Results have comforted the idea that ocean acidification alone does not lead to significant modifications in community structure and functioning and provided coherent results on group-specific production rates.

However, as the detection of these PLFA has sometimes been difficult in our samples, there is a strong need to adapt the experimental and analytical protocols to limit the uncertainties that remain with this technique in oligotrophic areas. First of all, it would be necessary to adapt filtration speeds and filters pore size with the purpose to improve the detection of bacterial PLFA that has proven difficult during our experiments. Furthermore, it would be necessary for future experiments to establish the PLFA composition of species or group of species (through culture experiments) commonly found in the Mediterranean Sea as well as to determine PLFA to carbon conversion factors specifically for this region. Using PLFA and carbon isotope analyses with labelling studies could then be used to investigate the carbon cycle in these regions in a quantitative (e.g. measure of primary production) and qualitative (e.g. food web links) way with details on the different plankton compartments.

This thesis has allowed filling a gap by performing experiments in a chronically understudied area (i.e. oligotrophic provinces) and under undisturbed nutrient level conditions. To complement experimental data acquired during this thesis, a literature survey has shown that the majority of the studies have reported no effect of ocean acidification alone on biomass and primary production. Although the amount of experiments focused on ocean acidification effect on plankton communities has drastically increased in the past few years, it

remains impossible to propose a general concept to understand and project in which conditions and at which locations this stressor will have an effect on plankton structure, biomass and production in future decades. In addition, very few studies performed at community level have considered ocean acidification in combination to other relevant drivers (e.g. ocean warming, nutrient availability, irradiance). The few multiple drivers experiments performed have shown that ocean acidification does not act as a primary driver, although possible interactions between these drivers are commonly reported.

At global scale, the fact that marine primary production seems mostly resilient to ocean acidification could have large consequences on global climate change. Indeed, in a situation in which plankton communities do not adapt to future high CO₂ conditions by fixing more carbon in surface layers, they will not help mitigating atmospheric CO₂ increase. In addition, ocean warming seems to favour small phytoplankton species that have lower carbon export efficiency as well as decrease solubility pump. These findings indicate that plankton communities might not help mitigating atmospheric CO₂ increase by enhanced biological carbon pump. In order to confirm these findings and project future structure and functioning of plankton communities, more time and research efforts are needed.

To do so, several methodological approaches can be used to investigate the effect of climate change on plankton communities and are complementary. All together, micro- and meso-cosmes, field studies, time series and modelling can provide clues to predict the effect of climate change through a strong and efficient international collaboration. For all of them, time is required (years or more exactly decades) that scientific community does not have and this is one of the most critical issues faced by the scientific community: research takes time while policy makers and funding agencies require quick answers. Furthermore, this survey on the effects of ocean acidification at community level is only a first step and efforts should be pursued to take into account carbon:nutrient supply, elemental composition, taxonomic composition and other parameters that characterise a plankton community. For this, more work is needed particularly to quantify the plankton community structure using mean cell size, abundances or other proxies that are not always available. This will provide a review of the current knowledge on ocean acidification effect at community level as shown by experimental work that could ultimately be compared to model projections.

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Appendix

Appendix A: Monitoring data acquired after Villefranche mesocosm experiment

Appendix B: Table with data from the literature survey

Appendix C: detailed *Curriculum Vitae*, list of publications, oral presentation and poster, budget of the thesis project

Context

As mentioned in chapter II, at the end of the mesocosm experiment we have decided to continue samplings in the bay during few more days in order to acquire high-frequency data during a bloom in this area. Indeed, when it was clear that the bags had been damaged by the storm and it was not possible to follow the experiment, levels of chlorophyll *a* increased in the Bay of Villefranche suggesting a potential bloom. The material was already set-up thus it was an opportunity to measure some parameters and metabolic rates in the Bay of Villefranche. The complete list of parameters and processes is presented in Table A-1.

The weekly-based monitoring at Point B in the Bay of Villefranche started in 1957 for core parameters such as temperature, nutrients and chlorophyll (see <http://somlit.epoc.u-bordeaux1.fr>) and more recently for pigments, flow cytometry and carbonate chemistry (see http://www.lav.obs-vlfr.fr/fr/missions_d_observation.html). However, data on primary production are much more scarce. Results on metabolic rates obtained during the mesocosm experiment in the Bay (referred to as OUT) using the ^{14}C method were complemented by this monitoring over a few days and provided an interesting time series. In addition, we have taken the opportunity of having all experimental set-ups available to perform a comparison of three different incubation methods to assess primary production rates: O_2 light-dark, ^{14}C and ^{13}C labelling

Table A-1. Parameters and processes measured during the monitoring

Parameter	Method and/or instrument	Name of the person the data belong to
T°, S, O ₂ , ...	CTD-Seabird SBE 19plus V2	F. Gazeau and C. Guieu
Irradiance	LI-192SA quantum sensor (LI-COR)	F. Gazeau and C. Guieu
Nitrogen and Phosphate	Nano-molecular detection method	C. Guieu and J. Louis
DIC	AIRICA	F. Gazeau and S. Alliouane
Alkalinity	Titration	F. Gazeau and S. Alliouane
Pigments	HPLC	F. Gazeau and C. Guieu
Cell abundance	Flow cytometry	M-L Pedrotti
Taxonomy	Uthermol	F. Gazeau and C. Guieu
^{14}C PP, DOC and CF	Micro-difusion Technique	L. Maugendre
O ₂ -LD	Winkler titration	F. Gazeau, M. Gaubert and L. Maugendre
^{13}C -PP	^{13}C measurment on EA-IRMS	L. Maugendre
Elemental composition	Elemental Analyser (EA)	L. Maugendre
TEP	Spectrophotometry	M-L Pedrotti

Material and Method

Samplings for the monitoring were performed before sunrise using a 5 L integrated sampler and a CTD for 6 days from March 13th to 18th 2013. Carbonate chemistry, pigments and metabolic rates presented here were sampled as described in Gazeau et al. (in prep, a) and in chapter II for oxygen light-dark (O₂-LD) and ¹⁴C production measurements. In addition to these methods already described, the ¹³C-PP method was used over 24 hours incubations (Hama et al. 1983). Details of the protocol follow.

In the laboratory, 500 mL of water was filtered through a pre-weighed and pre-combusted GF/F filter for initial POC and ¹³C-POC measurements. In 2 L seawater, 30 mg of 99 % ¹³C-sodium bicarbonate were added. A subsample of 20 mL for ¹³C-DIC analyses was immediately taken and spiked with 10 μL of HgCl₂ saturated solution. Labelled seawater was then transferred into three 2.5 L polycarbonate transparent bottles and in one dark bottle. Bottles were firmly closed and incubated for 24 h on the incubation line located next to the mesocosms with the O₂-LD and ¹⁴C bottles. Upon completion of the incubations, all bottles were removed and O₂-LD and ¹⁴C samples were treated as described in Chapter 2. For the ¹³C samples, seawater was filtered through pre-combusted and pre-weighed GF/F filters and dried at 50 °C. ¹³C-POC and ¹³C-DIC samples was then analysed as described in Chapter 3. Carbon uptake in the light and in the dark (in μmol C L⁻¹ d⁻¹) were then calculated following the equation of Hama et al. (1983):

$$Q = ((a_{is} - a_{ns}) / (a_{ic} - a_{ns})) \times (C / t) \times 24$$

where, a_{is} is the atomic % of ¹³C in the incubated sample (atomic % ¹³C-POC final); a_{ns} is the atomic % in the natural sample (initial); a_{ic} is the atomic % in the dissolved inorganic carbon; C is the final POC concentration in the incubated sample (μmol L⁻¹) and t is the incubation time (hours). Primary production was then obtained by removing dark incorporation to light incubation:

$$PP^* = Q_L - Q_D$$

with Q_L the light incorporation and Q_D the dark incorporation.

A discrimination factor (f) can also be taken into account to correct the difference in molecular weight between ¹³C and ¹²C, a value of 1.025 was used here (Hama et al. 1983). Corrected primary production is then expressed as:

$$PP = PP^* \times f$$

Results and Discussion

The purpose of this Appendix is not to fully describe and analyse the results obtained during this monitoring. Indeed, all data collected during these days are not presented here (nutrient, flow cytometry, TEPs etc) and will be integrated at a later stage in the frame of a collective study. Here, we will focus only on carbonate chemistry, chlorophyll *a*, pigments and metabolic rates.

Environmental conditions and metabolic rates dynamic

Carbonate chemistry was very constant during those days, with typical pH value of 8.10 pH unit and $p\text{CO}_2$ varying between 357 to 383 μatm (Table A-2). Chlorophyll *a* in the Bay was higher than during the mesocosm experimental period. Indeed, before March 4th, chl *a* was never higher than 0.94 $\mu\text{g Chl } a \text{ L}^{-1}$. During the monitoring, chl *a* slightly decreased from 1.26 to 1.10 $\mu\text{g Chl } a \text{ L}^{-1}$. This comforted the idea that the bloom did not start during the mesocosm experiment and the term “pre-bloom conditions” as used in chapter II is then justified.

Table A-2. Carbonate chemistry measured from dissolved inorganic carbon (DIC) and total alkalinity (TA) allowing calculation of the other parameters: pH, $p\text{CO}_2$, bicarbonate and carbonate ions, saturation state for aragonite (Ω_{Ar}) and calcite (Ω_{Ca}). Seacarb package (version 3.0) was used with Flag 15 (Lavigne, Epitalon and Gattuso 2014). A constant temperature of 13.2 °C and salinity of 37.8 psu were used.

Date	09/03/13	13/03/13	14/03/13	15/03/13	16/03/13	17/03/13	18/03/13
DIC ($\mu\text{mol kg}^{-1}$)	2286	2281	2291	2283	2287	2276	2281
TA ($\mu\text{mol kg}^{-1}$)	2559	2551	2558	2555	2559	2559	2558
pH _T	8.10	8.10	8.10	8.10	8.10	8.12	8.11
$p\text{CO}_2$ (μatm)	375	376	384	375	377	358	367
HCO_3^- ($\mu\text{mol kg}^{-1}$)	2077	2072	2084	2074	2078	2061	2069
CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	195	193	191	194	194	201	197
Ω_{Ar}	2.9	2.9	2.9	2.9	2.9	3.0	3.0
Ω_{Ca}	4.5	4.5	4.5	4.5	4.5	4.7	4.6

All production rates collected are presented in Figure A-1 and Table A-3. At the start of the monitoring, GPP-O₂ and NCP-O₂ presented higher values than during the mesocosm experiment and rates decreased over time (Figure A-1) as nutrient decreased. NCP-O₂ were always positive showing that the community was autotrophic.

¹⁴C-PP rates were also measured outside during the mesocosm experiment and were never higher than 0.80 μmol C L⁻¹ d⁻¹ while during the monitoring values higher to 1 μmol C L⁻¹ d⁻¹ were frequently measured (Figure A-2). As during the mesocosm experiment, ¹⁴C-PP rates were lower than oxygen-based rates (GPP-O₂) except at the end of the monitoring (Figure A-1). DO¹⁴C production increased during the monitoring and percentage of extracellular release (PER) increased from 9.9 to 33 %.

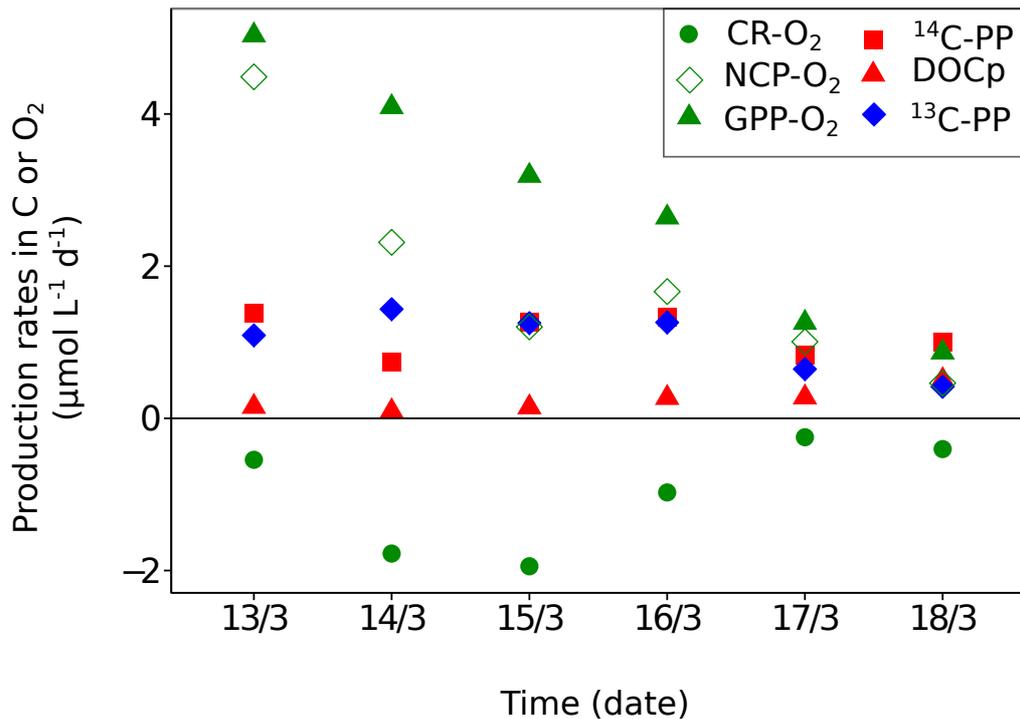


Figure A-1. Production rates measured with the oxygen light-dark technique (O₂-LD), ¹⁴C and ¹³C labelling techniques. In green (O₂-LD), net community production rates (NCP-O₂; empty diamonds), gross primary production (GPP-O₂; full triangles) and community respiration (CR-O₂; full circles). In red (¹⁴C), ¹⁴C particulate primary production (¹⁴C-PP; full squares) and dissolved production (DOCp; full triangles) and in blue the ¹³C particulate production (¹³C-PP; open diamonds).

Table A-3. Chlorophyll *a* (chl *a*) concentrations as well as all metabolic rates values collected during the monitoring. Particulate and dissolved production measured by ¹⁴C technique (¹⁴C-PP and DOCp, respectively); percentage of extracellular release (PER; PER = DOCp/(DOCp+¹⁴C-PP); calcification determined with ¹⁴C labelling (CF); net community production (NCP); community respiration (CR); gross primary production (GPP); particulate primary production with ¹³C incubation (¹³C-PP*) and corrected for differences in molecular weight (¹³C-PP).

Date	09/03/13	13/03/13	14/03/13	15/03/13	16/03/13	17/03/13	18/03/13
chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.85	1.26	1.13	1.12	1.04	1.00	1.10
¹⁴ C-PP ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	1.05	1.38	0.74	1.27	1.33	0.83	1.00
DOCp ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	0.12	0.15	0.09	0.15	0.27	0.28	0.50
PER (%)	10.00	9.87	14.49	10.27	21.37	24.90	33.40
CF ($\text{nmol C L}^{-1} \text{d}^{-1}$)	0.01	0.01	0.08	0.04	0.05	0.01	0.04
NCP ($\mu\text{mol O}_2 \text{L}^{-1} \text{d}^{-1}$)	-	4.49	2.31	1.24	1.67	1.01	0.46
CR ($\mu\text{mol O}_2 \text{L}^{-1} \text{d}^{-1}$)	-	-0.55	-1.78	-1.94	-0.97	-0.25	-0.40
GPP ($\mu\text{mol O}_2 \text{L}^{-1} \text{d}^{-1}$)	-	5.03	4.09	3.19	2.64	1.26	0.87
¹³ C light incorporation ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	-	1.12	1.45	1.26	1.27	0.66	0.42
¹³ C dark incorporation ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	-	0.06	0.05	0.04	0.04	0.03	0.01
¹³ C-PP* ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	-	1.06	1.40	1.22	1.23	0.63	0.40
¹³ C-PP ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	-	1.09	1.44	1.25	1.26	0.65	0.41

A shift in community structure occurred as observed by pigment contents. Indeed, small Haptophytes (based on 19' hexanoyloxyfucoxanthin pigment) increased in biomass while Diatoms (based on fucoxanthin) decreased. As samplings were performed before sunrise (around 6:30 am), PAR in the water column by CTD profile were not measured and extinction coefficient were not calculated. Instead, the mean surface irradiance during the incubations was used and varied during the monitoring from 40 to 280 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Only ^{14}C -PP rates significantly correlated with PAR (linear regression, $n = 6$, $r = 0.93$, $p < 0.05$; Figure A-3). ^{13}C -PP rates also showed an increasing trend with increasing PAR levels although not significant (Figure A-3).

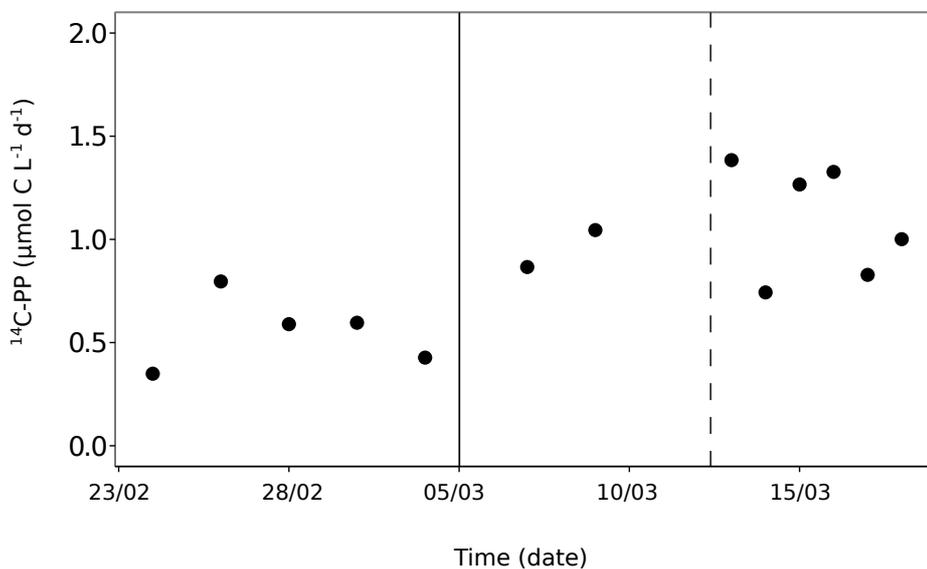


Figure A-2. Primary production rates in the Bay of Villefranche using the ^{14}C -PP technique: during the mesocosm experiment (before March 5th, full line), and during the monitoring (after dotted line).

Comparison of the different methods

The carbon-based measurements were closed despite there had some diverging data points due either relatively low ^{14}C (March 14th) or ^{13}C (March 18th) measurements. As a consequence of the small number of measurements, the correlation between ^{14}C and ^{13}C -PP was not significant ($r = 0.23$, $p > 0.05$). Increasing the number of measurement might improve this correlation. These first results are however encouraging for the use of ^{13}C as an alternative to radioactive ^{14}C even if the method needs perfection, especially in the determination of ^{13}C -DOC that remains technically difficult.

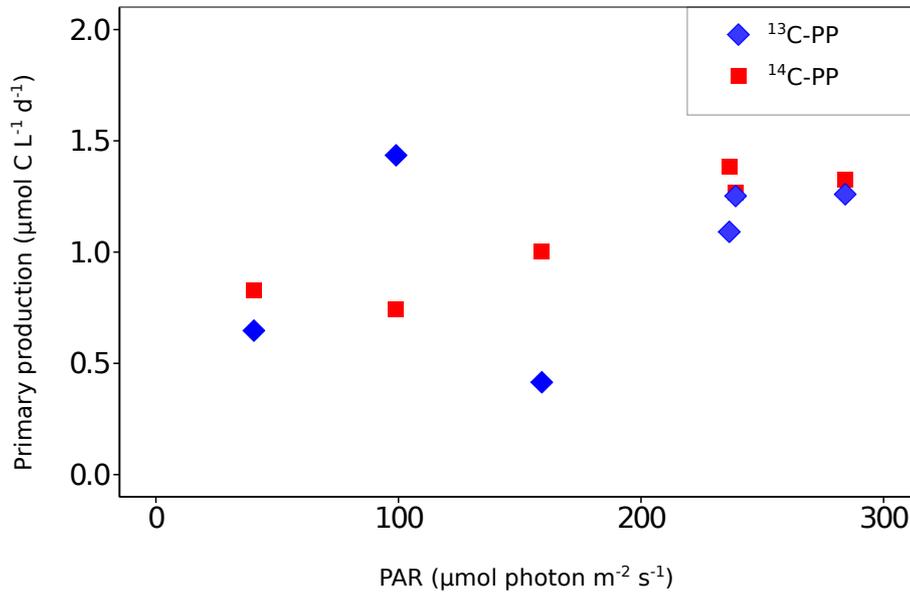


Figure A-3. Relation between PAR and production rates based on the ¹³C and ¹⁴C techniques.

Concluding remarks

Even if this analyse of the monitoring is not complete here, it has allowed to clarify two points:

- The mesocosm experiment **has been performed under pre-bloom conditions** as shown by chlorophyll *a* and ¹⁴C-PP measurements after the end of the experiment.
- The ¹³C-PP method **has provided results close to those obtained with ¹⁴C-PP** and the method was pretty simple to set-up and sample analyses are trivial as soon as the equipment is available.

As perspectives on this work:

- Primary production rates measured outside during this period have to be **related to nutrient concentrations** and all the **results collected outside the mesocosm during this winter-spring period could be related to the weekly Point B time series data**.
- A deeper **analysis of pigment concentrations** together with flow cytometry data would be interesting as it appears that the **community has rapidly shifted due to weather conditions**.
- Extra tests of the ¹³C-PP method **and improvements of the techniques to measure ¹³C-DOC should be performed to refine this method** that could replace to some extent the radioactive ¹⁴C-PP method.

Appendix B. Table with data collected from the literature used for the general discussion of the thesis (chapter V).

Location indicates geographical information's of the experiment. BATS for Bermuda Atlantic Time-series Study station; NW Med Sea for NW Mediterranean Sea (our experiments) and NW Europ Cont Shelf for NW European continental shelf (Richier et al. 2014)

Production measurement methods provide information on the method used for the measure of production. With ^{14}C -PP for primary production based on ^{14}C labelling with incubation time indicated in parenthesis; ^{18}O -GPP for gross primary production measured by ^{18}O labelling; O_2 -LD for the oxygen light-dark method providing net community production; photochemical quantum efficiency of Photosystem II (photo. quantum eff. PS II) as determined using Fast Repetition Rate fluorometer (FRRf) or by the pulse amplitude modulated (PAM) fluorometry; particulate organic carbon (POC) accumulation relative to chlorophyll *a* (chl *a*).

Site or experiment details to distinguish the different studies by the period, sites, the nutrient conditions, the iron (Fe) state or the name of the experiment (PeECE for Pelagic Ecosystem CO_2 Enrichment I, II and III).

Publications from which the data have been collected are indicated and references can be found in the Reference section of the thesis.

Incubation volume in litre; duration of the experiment in day; mean temperature (temp) in $^{\circ}\text{C}$ during the study; initial chlorophyll *a* (init chl *a*) in $\mu\text{g chl } a \text{ L}^{-1}$. Initial nitrogen (initial NO_x =nitrate+nitrite) and phosphate (initial PO_4) in $\mu\text{mol L}^{-1}$. $p\text{CO}_2$ is the initial $p\text{CO}_2$ level in μatm and relative $p\text{CO}_2$ is the initial $p\text{CO}_2$ level tested relative to the control. Response ratio calculated for chlorophyll *a* and primary production (RR-chl *a* and RR-PP respectively). The main phytoplankton groups reported in the study are indicated when available.

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp °C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
BATS, N Atlantic	¹⁴ C-PP (2-3h)	September 2009	Lomas et al. (2012)		2.8	28	0.05	5	0.5	800	2.5	1	1	<i>Synechococcus</i> and <i>Prochlorococcus</i> communities
Bay of Calvi, NW Med Sea	¹⁸ O-GPP (12h); O ₂ -LD; NCP- ¹³ C		Maugendre et al. in prep-a,b (Chapter II & III)	50000	20	24	0.06	0.05	0.02		1	1	1	Haptophytes and cyanobacteria (mostly <i>Synechococcus</i>)
NW Europ Cont Shelf	FRRf	site E5	Richier et al. (2014)	4	4	13.9	0.2	0.26	0.05	1000	2.6	0.16	0.88	Dinoflagellates, picoeukaryotes and <i>Synechococcus</i>
NW Europ Cont Shelf	FRRf	site E5	Richier et al. (2014)	4	4	13.9	0.2	0.26	0.05	750	2	0.2	0.9	Dinoflagellates, picoeukaryotes and <i>Synechococcus</i>
NW Europ Cont Shelf	FRRf	site E5	Richier et al. (2014)	4	4	13.9	0.2	0.26	0.05	550	1.5	0.83	1	Dinoflagellates, picoeukaryotes and <i>Synechococcus</i>
N Pacific	¹⁴ C-PP (6-9h)		Riebesell et al. (2000)		9	13	0.2	5		800	1		1	Coccolithophores
SW Atlantic transect	¹⁴ C-PP (2h)		Hein & Sand-Jensen (1997)		0.08		0.2			850	2		1.15	
SW Atlantic transect	¹⁴ C-PP (2h)		Hein & Sand-Jensen (1997)		0.08		0.2			1250	3		1.15	
N Pacific	POC/chl <i>a</i> production		Yoshimura et al. (2013)	12	14	9.2	0.21	16	1.4		1	1	1	70% ultraeukaryotes and 20% <i>Synechococcus</i>

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Southern coast of Japan			Hama et al. (2011)	500	15	12	0.25	12	1.4	800	1.9	0.17		Diatoms, cryptophytes, haptophytes, dinoflagellates and prasinophytes
Oshtock Sea, NW Pacific	POC accumulation		Yoshimura et al. (2010)	9	14	13.5	0.31	0.05	0.25	480	2.4	1	0.29	<i>Synechococcus</i> , picoeucaryotes and bacteria but few diatoms and dinoflagellates,
Oshtock Sea, NW Pacific	POC accumulation		Yoshimura et al. (2010)	9	14	13.5	0.31	0.05	0.25	590	2.9	1	0.29	<i>Synechococcus</i> , picoeucaryotes and bacteria but few diatoms and dinoflagellates,
Oshtock Sea, NW Pacific	POC accumulation		Yoshimura et al. (2010)	9	14	13.5	0.31	0.05	0.25	280	1.4	1		<i>Synechococcus</i> , picoeucaryotes and bacteria but few diatoms and dinoflagellates,
BATS, N Atlantic	¹⁴ C-PP (2-3h)	March 2010	Lomas et al. (2012)		3.2	19	0.34	5	0.5		1	1	1	<i>Prochlorococcus</i> communities
N Pacific	PAM	Fe limited experiment	Endo et al. (2013)	12	14	14	0.34	14	1.2		1	1	1	Diatoms, haptophytes and <i>Synechococcus</i>

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp °C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Bering Sea, N Pacific	POC/chl <i>a</i> production		Yoshimura et al. (2013)	12	14	8.4	0.39	16	1.5	600	2	0.7	0.5	Dominant diatoms (65 %)
Bering Sea, N Pacific	POC/chl <i>a</i> production		Yoshimura et al. (2013)	12	14	8.4	0.39	16	1.5	960	3.2	0.9	0.7	Dominant diatoms (65 %)
Bering Sea, N Pacific	POC/chl <i>a</i> production		Yoshimura et al. (2013)	12	14	8.4	0.39	16	1.5	1190	4	1	0.7	Dominant diatoms (65 %)
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	nutrient replete	Schulz et al. (2013); Engel et al. (2013)	50000	7	2	0.47	5.5	0.4	345	1.93	0.76	0.3	Pico- and nano-plankton
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	nutrient replete	Schulz et al. (2013); Engel et al. (2013)	50000	7	2	0.47	5.5	0.4	254	1.42	0.76	0.47	Pico- and nano-plankton
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	nutrient replete	Schulz et al. (2013); Engel et al. (2013)	50000	7	2	0.47	5.5	0.4	667	3.74	1.09	1.74	Pico- and nano-plankton
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	nutrient replete	Schulz et al. (2013); Engel et al. (2013)	50000	7	2	0.47	5.5	0.4	595	3.34	1.15	0.36	Pico- and nano-plankton
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	nutrient replete	Schulz et al. (2013); Engel et al. (2013)	50000	7	2	0.47	5.5	0.4	423	2.37	1.18	1.26	Pico- and nano-plankton
Svalbard, Arctic Ocean	O ₂ -LD	nutrient replete	Tanaka et al. (2013)	50000	7	2	0.47	5.5	0.4	667	3.74		0.57	Pico- and nano-plankton
Svalbard, Arctic Ocean	O ₂ -LD	nutrient replete	Tanaka et al. (2013)	50000	7	2	0.47	5.5	0.4	345	1.93		0.73	Pico- and nano-plankton

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp °C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO_x $\mu\text{mol L}^{-1}$	Initial PO_4 $\mu\text{mol L}^{-1}$	Initial $p\text{CO}_2$ μatm	Relative $p\text{CO}_2$	RR-chl <i>a</i>	RR-PP	Main community composition
Svalbard, Arctic Ocean	O ₂ -LD	nutrient replete	Tanaka et al. (2013)	50000	7	2	0.47	5.5	0.4	254	1.42		0.85	Pico- and nano-plankton
Svalbard, Arctic Ocean	O ₂ -LD	nutrient replete	Tanaka et al. (2013)	50000	7	2	0.47	5.5	0.4	423	2.37		0.1	Pico- and nano-plankton
Svalbard, Arctic Ocean	O ₂ -LD	nutrient replete	Tanaka et al. (2013)	50000	7	2	0.47	5.5	0.4	595	3.34		0.49	Pico- and nano-plankton
Bering Sea, N Pacific	Photo. quantum eff. PS II	Fe added experiment	Suggie et al. (2013)	12	8	8.2	0.5				1	1	1	Diatoms and dinoflagellates
Bergen, Norway		PeECE II (2003)	Engel et al. (2008)	20000	20	10	0.5	9	0.5		1	1		Several diatoms species and some <i>Emiliana huxleyi</i>
Bergen, Norway	¹⁴ C-PP (4h)	PeECE II (2003)	Egge (unpublisehd)	20000	20	10	0.5	9	0.5		1		1	Several diatoms species and some <i>Emiliana huxleyi</i>
Bergen, Norway	O ₂ -LD	PeECE II (2003)	Engel (unpublished)	20000	20	10	0.5	9	0.5		1		1	Several diatoms species and some <i>Emiliana huxleyi</i>
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	before nutrient addition	Schulz et al. (2013); Engel et al. (2013)	50000	9	2	0.62	0.03	0.05	368	2.01	0.88	0.58	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	¹⁴ C-PP (24h)	before nutrient addition	Schulz et al. (2013); Engel et al. (2013)	50000	9	2	0.62	0.03	0.05	269	1.47	1.05	1.79	Nanophytoplankton and haptophytes

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO_x $\mu\text{mol L}^{-1}$	Initial PO_4 $\mu\text{mol L}^{-1}$	Initial $p\text{CO}_2$ μatm	Relative $p\text{CO}_2$	RR-chl <i>a</i>	RR-PP	Main community composition
Svalbard, Arctic Ocean	^{14}C -PP (24h)	before nutrient addition	Schulz et al. (2013); Engel et al. (2013)	50000	9	2	0.62	0.03	0.05	476	2.6	1.14	1.55	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	^{14}C -PP (24h)	before nutrient addition	Schulz et al. (2013); Engel et al. (2013)	50000	9	2	0.62	0.03	0.05	681	3.72	1.87	1.61	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	O_2 -LD	before nutrient addition	Tanaka et al. (2013)	50000	9	2	0.62	0.03	0.05	681	3.72		1	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	O_2 -LD	before nutrient addition	Tanaka et al. (2013)	50000	9	2	0.62	0.03	0.05	476	2.6		1	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	O_2 -LD	before nutrient addition	Tanaka et al. (2013)	50000	9	2	0.62	0.03	0.05	269	1.47		1	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	O_2 -LD	before nutrient addition	Tanaka et al. (2013)	50000	9	2	0.62	0.03	0.05	368	2.01		1	Nanophytoplankton and haptophytes
Svalbard, Arctic Ocean	^{14}C -PP (24h)	nutrient depleted-post bloom	Schulz et al. (2013); Engel et al. (2013)	50000	6	5	0.65	2.1	0.1	586	3.4	0.97	2.65	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	^{14}C -PP (24h)	nutrient depleted-post bloom	Schulz et al. (2013); Engel et al. (2013)	50000	6	5	0.65	2.1	0.1	396	2.29	1.05	2.87	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	^{14}C -PP (24h)	nutrient depleted-post bloom	Schulz et al. (2013); Engel et al. (2013)	50000	6	5	0.65	2.1	0.1	237	1.37	1.06	1.42	Presence of Diatoms and Dinoflegellates

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO_x $\mu\text{mol L}^{-1}$	Initial PO_4 $\mu\text{mol L}^{-1}$	Initial $p\text{CO}_2$ μatm	Relative $p\text{CO}_2$	RR-chl <i>a</i>	RR-PP	Main community composition
Svalbard, Arctic Ocean	^{14}C -PP (24h)	nutrient depleted-post bloom	Schulz et al. (2013); Engel et al. (2013)	50000	6	5	0.65	2.1	0.1	314	1.82	1.07	0.62	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	^{14}C -PP (24h)	nutrient depleted-post bloom	Schulz et al. (2013); Engel et al. (2013)	50000	6	5	0.65	2.1	0.1	540	3.13	1.25	2.76	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	O_2 -LD	nutrient depleted-post bloom	Tanaka et al. (2013)	50000	6	5	0.65	2.1	0.1	396	2.29		1.18	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	O_2 -LD	nutrient depleted-post bloom	Tanaka et al. (2013)	50000	6	5	0.65	2.1	0.1	586	3.4		0.92	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	O_2 -LD	nutrient depleted-post bloom	Tanaka et al. (2013)	50000	6	5	0.65	2.1	0.1	540	3.13		1.18	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	O_2 -LD	nutrient depleted-post bloom	Tanaka et al. (2013)	50000	6	5	0.65	2.1	0.1	237	1.37		1.35	Presence of Diatoms and Dinoflegellates
Svalbard, Arctic Ocean	O_2 -LD	nutrient depleted-post bloom	Tanaka et al.. (2013)	50000	6	5	0.65	2.1	0.1	314	1.82		1.12	Presence of Diatoms and Dinoflegellates
BATS, N Atlantic	^{14}C -PP (2-3h)	April 2010	Lomas et al. (2012)		1.5	19	0.69	5	0.5		1	1	1	<i>Synechococcus</i> and <i>Prochlorococcus</i> communities
NW Europ Cont Shelf	FRRf	site E3	Richier et al. (2014)	4	4	15.3	0.75	0.56	0.06		1	1	1	Dinoflagellates and <i>Synechococcus</i>

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Bering Sea, N Pacific	Photo. quantum eff. PS II	Fe limited experiment	Suggie et al. (2013)	12	8	8.2	1	18.1	1.47	600	1.6	0.5	0.88	Diatoms and dinoflagellates Initially <i>Synechococcus</i> with a temporal shift to <i>Emiliana huxleyi</i>
Bergen, Norway	O ₂ -LD; 14C-PP (24h)	PeECE I (2001)	Delille et al. (2005); Engel et al. (2005)	11000	19	11	1	15.3	0.5		1	1	1	Diatoms and dinoflagellates
Tasmania Bay of Villefranche, NW Med Sea	PAM ¹⁴ C-PP (24h); O ₂ -LD; NCP- ¹³ C		Nielsen et al. (2012) Maugendre et al. in prep-a,b (Chapter II & III)	2.5 50000	14 11	16 13	1 1.1	0.2 0.13	0.5 0.01		1 1	1 1	1 1	Haptophytes, Cryptophytes and <i>Synechococcus</i>
Godvari River, India	O ₂ -LD	low DIP	Biswas et al. (2011)	5.6	5	28	1.2	6.91	0.55	650	2	2	1.23	Diatoms and cyanobacteria Dinoflagellates, cryptophytes and <i>Synechococcus</i>
NW Europ Cont Shelf	FRRf	site E4	Richier et al. (2014)	4	4	14.6	1.5	0.87	0.12	550	1.4	1	1.1	Initially diatoms and <i>Emiliana huxleyi</i> with temporal shift to flagellates and cyanobacteria
Bergen, Norway	NCP-C _T	PeECE III (2005)	Riebeselle et al. (2007); Bellerby et al. (2008)	27000	25	10	1.5	14	0.7	700	2	1	1.41	

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Bergen, Norway	NCP-C _T	PeECE III (2005)	Riebeselle et al. (2007); Bellerby et al. (2008)	27000	25	10	1.5	14	0.7	1050	3	1	1.75	Initially diatoms and <i>Emiliana huxleyi</i> with temporal shift to flagellates and cyanobacteria
NW Europ Cont Shelf	FRRf	site E4	Richier et al. (2014)	4	4	14.6	1.5	0.87	0.12	750	1.8	1.7	1.3	Dinoflagellates, cryptophytes and <i>Synechococcus</i>
NW Europ Cont Shelf	FRRf	site E4	Richier et al. (2014)	4	4	14.6	1.5	0.87	0.12	1000	2.5	2.2	1.3	Dinoflagellates, cryptophytes and <i>Synechococcus</i>
Bergen, Norway	O ₂ -LD	PeECE III (2005)	Egge et al. (2009)	27000	25	10	1.5	14	0.7		1		1	Initially diatoms and <i>Emiliana huxleyi</i> with temporal shift to flagellates and cyanobacteria
Bergen, Norway	¹⁴ C-PP (4h)	PeECE III (2005)	Egge et al. (2009)	27000	25	10	1.5	14	0.7	700	2		1.15	Initially diatoms and <i>Emiliana huxleyi</i> with temporal shift to flagellates and cyanobacteria

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp °C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Bergen, Norway	¹⁴ C-PP (4h)	PeECE III (2005)	Egge et al. (2009)	27000	25	10	1.5	14	0.7	1050	3			Initially diatoms and <i>Emiliana huxleyi</i> with temporal shift to flagellates and cyanobacteria
Godvari River, India	O ₂ -LD	high DIP	Biswas et al. (2011)	5.6	5	28	2.34	7.58	3.2	244	1.5	1.22	0.86	Diatoms and cyanobacteria
Godvari River, India	O ₂ -LD	high DIP	Biswas et al. (2011)	5.6	5	28	2.34	7.58	3.2	363	2	1.4	1.57	Diatoms and cyanobacteria
Denmark	¹⁴ C-PP (2h)		Nielsen et al. (2010)	2.5	14	17.9	2.5	0.65	0.18		1	1	1	Diatoms, cryptophytes, prasinophytes and dinoflagellates
NW Europ Cont Shelf	FRRf	site E1	Richier et al. (2014)	4	4	11.3	3.5	1.06	0.09	550	1.5	1.06	1	Dinoflagellates and cryptophytes
NW Europ Cont Shelf	FRRf	site E1	Richier et al. (2014)	4	4	11.3	3.5	1.06	0.09	750	2	1.2	1	Dinoflagellates and cryptophytes
NW Europ Cont Shelf	FRRf	site E1	Richier et al. (2014)	4	4	11.3	3.5	1.06	0.09	1000	2.8	1.3	1	Dinoflagellates and cryptophytes
NW Europ Cont Shelf	FRRf	site E2	Richier et al. (2014)	4	4	11.8	3.8	0.28	0.14		1	1	1	Diatoms and dinoflagellates
Denmark	¹⁴ C-PP (2h)		Nielsen et al. (2010)	2.5	14	10.7		1.05	0.27		1	1	1	Diatoms, cryptophytes, prasinophytes and dinoflagellates

Location	Production measurement method	Site or experiment details	Publications	Volume L	Duration day	Temp ° C	Initial chl <i>a</i> $\mu\text{g chl } a \text{ L}^{-1}$	Initial NO _x $\mu\text{mol L}^{-1}$	Initial PO ₄ $\mu\text{mol L}^{-1}$	Initial <i>p</i> CO ₂ μatm	Relative <i>p</i> CO ₂	RR-chl <i>a</i>	RR-PP	Main community composition
Denmark	¹⁴ C-PP (2h)		Nielsen et al. (2010)	2.5	14	10.7		1.05	0.27		1	1	1	Diatoms, cryptophytes, prasinophytes and dinoflagellates
Ross sea, S Ocean	¹⁴ C-PP (24h)		Tortell et al. (2008)	4	14	0				380	3.8		1.25	Diatoms
Weddel Sea, S Ocean	¹⁴ C-PP (24h)	Fe limited experiment	Hoppe et al. (2013)	4	24	2		29	2		1		1	Diatom-dominated assemblage
Weddel Sea, S Ocean	¹⁴ C-PP (24h)	Fe added experiment	Hoppe et al. (2013)	4	24	2		29	2	390	2		1.4	Diatom-dominated assemblage
Weddel Sea, S Ocean	¹⁴ C-PP (24h)	Fe added experiment	Hoppe et al. (2013)	4	24	2		29	2	800	4		1.9	Diatom-dominated assemblage

Laure MAUGENDRE

Born September 22 1988 (26); Vannes (Morbihan, France)

Current professional contact

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Permanent personal contacts

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56000 ARRADON
+33 6 32 41 06 19
laure.maugendre@gmail.com

Research interest: Ocean; carbon cycle; phytoplankton; impact of human activities

Research related interest: ethical issues on climate change research; science with society

UNIVERSITY EDUCATION

Helsinki University, Finland

14-18 July 2014: League of European Research Universities 5th summer school

“Doing the right things right-Research integrity in a complex society”.

*Laboratoire d’Océanographie de Villfranche - UMR 7093
Sorbonne Universités-Université Pierre et Marie Curie*

2011-now: PhD in Biological Oceanography, defense October 31st 2014.

« Response of plankton community to ocean warming and acidification in the NW Mediterranean Sea »

Supervisors : Drs. Frédéric Gazeau and Jean-Pierre Gattuso

Institut Universitaire Européen de la Mer (IUEM/UBO)

2009-2011: Master’s degree in marine environmental chemistry. With honors.

Université de Bretagne Occidentale Brest (UBO)

2006-2009: B. Sc. in chemistry.

Last semester performed in A Coruña (Spain) during an Erasmus exchange.

First year of bachelor in biology. With distinctions.

RESEARCH EXPERIENCE

*National Oceanographic Center (NOC-SOTON)
Southampton, Royaume-Uni*

2011 January-June: Second Master’s degree internship, Ocean biogeochemistry and ecology group.

Subject: *Comparative Physiology of Coccolithophores Species.* Comparison of three different morphotypes of strains of *Emiliania huxleyi* grown in laboratory under different Nitrogen:Phosphate ratios and comparison to other coccolithophores species.

Supervisor: Dr Alex Poulton

*Instituto de Investigación Marinas (IIM-CSIC)
Vigo, Espagne*

2010 January-February: First Master's degree internship, Marine biogeochemistry group.

Subject: Processing preliminary results of sediment metal content in a coastal lagoon and data visualisation for a seminary. Use of atomic absorption methods (ETAAS and FAAS) and field trip to the "Laguna de Louro" for sediment sampling during 2 days.

Supervisor: Dr Ricardo Prego

*Université de Bretagne Occidentale (UBO)
Brest, France*

2008 July: Bachelor's Degree voluntary internship, Marine lipids group.

Subject: Lipids extraction and analyses in sea shell.

Supervisor: Dr. Fabienne Legrand.

KNOWLEDGE TRANSMISSION

Students' supervision

2013: Co-supervision with F. Gazeau of Margaux Gaubert Second year of Master of Oceanography, UPMC-Paris 6 (6 months). Effect of ocean acidification on plankton metabolism.

Public education and outreach

2012 to 2014: Co-realisation « Ocean acidification» topic for *Mon Océan et Moi* website. Creation and Webmaster of 2 blogs for the scientific mission MedSeA Mesocosms Stareso (<http://medseastareso2012.obs-vlfr.fr>) and Villefranche (<http://medseavillefranche2013.obs-vlfr.fr>).

2014 May: Preparation and presentation of "High CO₂ world" for an audience of sub-divers of French federation (FFESSM) in the frame of a course in marine biology.

2013 October: Organisation and animation of the workshop « Ocean acidification» for the science festival in the laboratory.

2013 June: Organisation and animation of the workshop « Ocean acidification» for the Researchers's spring in Nice.

Teaching activities

Being located away from my university no teaching have been performed.

August 29th 2014: Animation of a discussion and debate on Science and society, Ethical issues in research within IMMERSION's course titled: Seas and Marine Organisms at the convergence between Philosophy and Biology.

SCIENTIFIC PUBLICATIONS

Manuscript accepted in a peer-reviewed journal:

Accepted in ICES Journal of Marine Science, August 2014.

Maugendre L., Gattuso J.-P., Louis J., De Kluijver A., Marro S., Soetaert K. and Gazeau F.
Effect of ocean warming and acidification on a plankton community in the NW Mediterranean Sea.

Manuscripts in preparation for peer-review journal (submission in a special issue for MedSeA mesocosm in Estuarine, Coastal and Shelf Science): (2/5 as first author)

- *Maugendre L., Gattuso J.-P., Poulton A., Delisanti W., Gaubert M. and Gazeau F.*
No effect of ocean acidification on planktonic metabolism in the NW oligotrophic Mediterranean Sea: results from two mesocosm studies. (submission early October 2014)
- *Maugendre L., Gattuso J.-P., De Kluijver A., Soetaert K., van Oevelen D., Middelburg J. J. and Gazeau F.*
Carbon-13 labelling studies show no effect of ocean acidification on Mediterranean plankton communities. (submission early November 2014)
- *Gazeau F., Sallon A., Lejeune P., Gobert S., Maugendre L., Louis J., Alliouane S., Taillandier V., Louis F., Obolensky G., Grisoni J.-M., Delissanti W. and Guieu C.*
First mesocosm experiments to study the impacts of ocean acidification on the plankton communities in the NW Mediterranean Sea (MedSeA project). (submission date: 01/10/2014)
- *Gazeau F., Sallon A., Maugendre L., Giani M., Celussi M., Michel L, Gobert S. and Borges A.V.* Impact of elevated CO₂ on pelagic production and carbon fluxes in an Mediterranean mesocosm study. (submission date: 01/02/2015)
- *Gazeau F., Guieu C., Rees A. Celussi M., Maugendre L., Pitta P. and other people (under discussion)*
Resilience of plankton communities to ocean acidification in a low nutrient low chlorophyll (LNLC) area. (will be the synthesis of the mesocosm experiments of the special issue)

International conferences:

Poster at the "Third Symposium on the Ocean in a High CO₂ World". September 2012, Monterey, California, USA.

Maugendre L., Gattuso J.-P. and Gazeau F.

Effects of ocean acidification and warming on a natural planktonic community in Mediterranean Sea.

Oral presentation in 'IMBER Open Science Conference'. June 2014, Bergen, Norway.

Maugendre L., Gattuso J.-P., Louis J., De Kluijver A., Marro S., Soetaert K. and Gazeau F.

Effect of ocean warming and acidification on a plankton community in the NW Mediterranean Sea.

IMPLICATION IN LABORATORY AND UNIVERSITY

Student representative of the Observatoire Océanologique de Villefranche (2012-2014).

Student representative of the doctoral school 129 (2014-2014).

Co-initiation of student seminars in the Laboratoire d'Océanographie de Villefranche (LOV) which are no organised by the representative students of the LOV.

Initiation and head of the organisation team (budget, speaker invitation, communication, ...) of the first edition of the Young Researcher Day in the Observatoire Océanologique de Villefranche. This day aim to reunite people (research, student, technician) from the two laboratory of the OOV as well as people external to the OOV with topic: Science what do you think about it? With the presence of Gilles Boeuf and Christian Sardet.

SKILLS

Initiative spirit, investment, contribute, linking people and disciplines, transfer of knowledge, advice, animate, sailing

Autonomy, human and material planification, relation with partners, realisation and monitoring budget

Writing skills: scientific papers; application for funding agencies

Scientific methods: carbonate chemistry calculation (Seacarb method); dissolved inorganic carbon measurement with AIRICA analyser; alkalinity measurement by titration; dissolved oxygen measurement by titration; dissolved organic carbon sampling and analysis; lipids extractions; stable isotope analysis using mass spectrometers; carbon radioactive manipulation and authorisation

MISCELLANEOUS

Scientific expertise

Reviewing of a scientific article for Biogeosciences Discussion (2014)

English – read, written and spoken (B2 level of the CECRL)

Spanish – good oral and written comprehension, good oral expression

OTHER WORKING EXPERIENCES

*Departmental Laboratory of analysis IDHESA
Plouzané, France*

2010 August: laboratory technician,

Physico-chemical analysis of drinking waters, swimming pools, seawaters (summer period quality control)

*Yacht Club of St Pierre Quiberon
Saint-Pierre Quiberon, France*

2009 August: Secretary,

Reception, enrolment, accounts,

*Information office
Arradon, France*

2008 August: Tourism information office,

Tourist' reception and advices on the activities available in the region (Brittany)

*Departmental Laboratory of analysis
Saint Avé, France*

2007 July-August: laboratory technician

Department of bacteriology: analysis of drinking water, swimming pools water, seawater (summer period quality control).

BUDGET OF THE THESIS PROJECT

Nature of costs		Amount (TTC €)	
Human resources	PhD candidate salary		51000
	Salary of the person in charge of the mesocosm organisation		NC*
	Salary for technician in Corsica		NC*
	Salary for technician in Villefranche		NC*
	Master internship in Corsica		NC*
	Master internship in Villefranche		2400
Computer material	Laptop and screen		1670
	Hard drive		145
Campaigns	Corsica (passenger ferry, food, accomodation) for 1 person		4922
Travels	Yerseke (The Netherlands)	December 2011	900
		May 2012	1000
		November 2012	725
		June 2013	500
		March 2014	500
	Leuven (Belgium)	May 2012	765
		August 2012	1800
	Southampton (UK)	May 2012	1000
		Travel of Dr Alex Poulton for 14C technique training	February 2013
	Conferences	Monterey (USA)	September 201
Bergen (Norway)		June 201	1000
Trainings	MedSeA workshop (Athens, Greece)	September 201	1000
	Training in Paris at the doctoral school insitute	November 2013	127
		March 2014	146
	Summer schools	Villefranche sur Mer - July 201	100
		Helsinki (Finland) - July 2014	1500
Chemical products	Carbon 13		8620
	Carbon 14		3920
	Oxygen 18		3770
	Others		720
Analyses and shipr	Carbon 13 and biomarkers	Yerseke	8800
	Oxygen 18	Leuven	3630
	Carbon 13-DOC	Canada	2425
Consumables			12690
		Total	118655
		Distribution financial burden	€ %
		UPMC	52373 44.2
League of European Reseach University (summer school july 2014)		500	0.4
MedSeA and eFOCE projects, LOV and supervisors research grants		65782	55.4

NC* information not communicated and which were part of the MedSeA mesocosm project lead by the LOV therefore related to the thesis project

FLYER OF THE YOUNG RESEARCHER DAY

ÇA Y EST C'EST LA RENTRÉE ...
MAIS AU FAIT ...

**QU'EST CE QUE LA SCIENCE
D'AUJOURD'HUI ??!!**

LES RYTHMES DE VIE S'ACCÉLÈRENT FAUT-IL
AUSSI ACCÉLÉRER LE RYTHME DES PUBLICATIONS ?

LES NOUVEAUX DÉFIS SOCIÉTAUX PEUVENT-ILS
S'APPLIQUER À LA RECHERCHE ?

NOUVELLES TECHNOLOGIES POUR LE TRANSFERT
DES SAVOIRS À LA SOCIÉTÉ: UN ART, UNE
NÉCESSITÉ, UNE OPPORTUNITÉ ?

AVEC LA PARTICIPATION SPÉCIALE DE:
GILLES BOEUF (PRÉSIDENT DU MNHN)
CHRISTIAN SARDET (DR, CNRS)

PARTAGEONS ENSEMBLE NOTRE REGARD
DE LA SCIENCE AU CŒUR DE LA SOCIÉTÉ

*Venez nombreux !
 Les Jeunes Chercheurs de l'OOV*



**LA SCIENCE AU
CŒUR DE LA SOCIÉTÉ**

**QUEL REGARD
PORTEZ VOUS ?**

ja2c
jeunes
chercheurs



**VENDREDI,
5 SEPTEMBRE 2014**

**À L'OBSERVATOIRE OCÉANOLOGIQUE DE
VILLEFRANCHE-SUR-MER**

DE 9H À 17H, SALLE TRÉGOUBOFF