The combined effect of ocean acidification and temperature increase on planktonic communities in the E. Mediterranean: a mesocosm experiment

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Abstract

Warming and acidification of the oceans are two major drivers of climate change. A large scale mesocosm experiment, focusing on the study of the synergistic impact of warming and acidification on the planktonic food web of the Eastern Mediterranean, was taken place at the mesocosm facilities of HCMR in Crete. Two different pCO_2 (present day and predicted for year 2100) were applied in triplicate and tested in two different temperatures (ambient seawater T-25^{o}C and ambient T plus 3^{o}C). Changes occurred at the level of phytoplankton biomass, under acidified conditions whereas the effect of warming was more evident on productivity. Furthermore, the influence of seawater pH and temperature on heterotrophic prokaryotes, diatoms, dinoflagellates and ciliates within the microbial food web is discussed.

Keywords: warming, ocean acidification, mesocosm experiment, E. Mediterranean Sea, microbial food web

1. Introduction

Increasing atmospheric CO_2 is causing major changes in seawater chemistry, referred to as ocean acidification. It is expected to have direct and indirect effects on the growth and physiological processes of a range of marine organisms, however it is unclear what will be the effect on planktonic food webs. Riebesell et al. (2007) reported a variety of biological and biogeochemical processes in the oceans with consequences on the community and ecosystem level. Changes will occur at the level of the primary producers (Rost et al. 2008; Egge et al. 2009), as well as heterotrophic prokaryotes/or consumers (Aberle et al. 2013).

To date only few data exist on planktonic community response to ocean acidification in oligotrophic, low nutrient-low chlorophyll waters, that represent a large part of the ocean (Gazeau et al. 2015) and none on the combined effect with temperature increase. In oligotrophic systems planktonic communities are constrained by nutrient limitation and no significant effect on gross primary production or community production was found under acidified conditions (Maugendre et al. 2015). To this end the present study aimed at understanding how marine planktonic communities may evolve in response to the combined impact of temperature and pCO_2 increase in the oligotrophic E. Mediterranean and focused on primary and heterotrophic bacterial production and the resultant standing stocks/composition of microbial communities (prokaryote, diatom, dinoflagellate, ciliate populations). Microbial communities are key players for estimates of the carbon balance in the future ocean. Direct changes at the bottom of the food web may be the result of shifts in plankton community composition and food web functioning.

2. Materials and methods

The mesocosm experiment was performed at the HCMR land-based mesocosm facilities in Crete, (CRETACOSMOS htpp://mesocosm.eu/cretacosmos), during late August/early September 2013, with no nutrient addition in order to simulate the ultra-oligotrophic environment of the E. Mediterranean (orthophosphate varied from 1.6 -11.5 nM and nitrate from 0.05-0.25μM (Tsapakis pers. comm.)

In total 12 mesocosms of 3 m^3 were deployed in two large concrete tanks for 2 weeks. Two different pCO_2 levels (present day and predicted for year 2100) were applied in triplicate. This was tested in two different temperatures (ambient seawater T-25^{o}C) and ambient T plus 3^{o}C).

Temperature was controlled by sophisticated, automated systems (Krasakopoulou et al. 2015). The experimental design provided the opportunity to test simultaneously the effects of ocean acidification and warming simulating per three mesocosms the current temperature and pCO_2 conditions (also served as control) as well as the Ocean Acidification (OA), Warming (W) and Green House conditions (OA and W).

Seawater samples from all mesocosms were collected every morning into polycarbonate bottles. Samples for Chlorophyll *α* (Chl *α*) were subsequently filtered through a 2.0 µm followed by a 0.2 µm polycarbonate filter (47 mm). Chl *a* concentrations were determined according to Holm-Hansen et al. (1965), using a TURNER TD 700 fluorometer and after extraction with 90% acetone. Primary production rates (PP) were estimated by means of the ^{14}C technique of Steemann-Nielsen (1952). Heterotrophic Bacterial Production (BP) was estimated by the ^{3}H-leucine method as described in detail in Van Wambeke et al. (1997). Abundance of *Synechococcus*, autotrophic pico-eukaryotes, heterotrophic prokaryotes and viruses was estimated with flow cytometry, in a FACS Calibur instrument. Samples for *Synechococcus* and autotrophic pico-eukaryotes were analyzed, within a few hours after collection, with no fixation/staining step and were discriminated according to their auto-fluorescence. Samples for heterotrophic prokaryotes were processed according to Marie et al. (1999). Diatoms, dinoflagellates and ciliates were counted under an inverted microscope (Olympus IX70) with the Utermöhl method.

3. Results

The effect of acidification was evident in Chl *a* concentration; there was a rise in all mesocosms until day 5 with a clear differentiation (ANOVA, $p<0.05$) between the acidified and the non-acidified treatments (Fig. 1a), mostly driven by the 0.2-2µm pico-sized fraction (data not shown). After the initial peak, Chl *a* levels declined again to almost starting concentrations, while after day 10 the concentration started to slightly increase, mainly in the acidified mesocosms (not statistically significant difference).

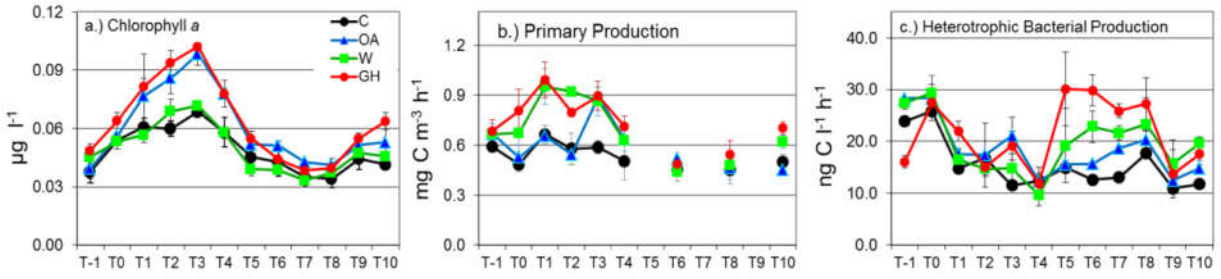

Fig. 1. a.) Total Chl *a* concentration (µg l^{-1}) b.) Primary production (mg C m^{-3} h^{-1}) c.) Bacterial Heterotrophic Production (ngC $l^{-1}h^{-1}$) through the experiment, under Present (C), Acidified (OA), Warming (W) and Green House (GH) conditions.

The evolution of primary and heterotrophic bacterial production during the days of the experiment (Fig. 1b, c) shows that rates are likely more dependent on temperature. An increase of PP was observed under warm conditions as compared to the control for the first four days of the experiment, followed by an increase of the same level in the acidified mesocosms at the 5th day. For the whole duration of the experiment, GH and W were significantly different from C (ANOVA, $p<0.05$) while OA was not ($p=0.8$). BP showed a small variation but when regarding specific time points an effect of pCO_2 was found. After day 6 an increase in BP rates was observed in the GH and W mesocosms followed by a lower increase in OA mesocosms, as a result after the increased PP levels, recorded during the 6 first days of the experiment.

Abundance of heterotrophic prokaryotes did not differ among treatments, throughout the experimental period. *Synechococcus* showed increased concentration at W, OA and GH treatments compared to control during the acidification period up to day 2, while during the whole experiment concentration was higher under warm conditions (ANOVA, $p<0.05$). Autotrophic pico-eukaryotes showed a two-fold increase during the acidification period (T-1 – T1) and a second but lower peak on T10, consistent among all treatments (Fig. 2).

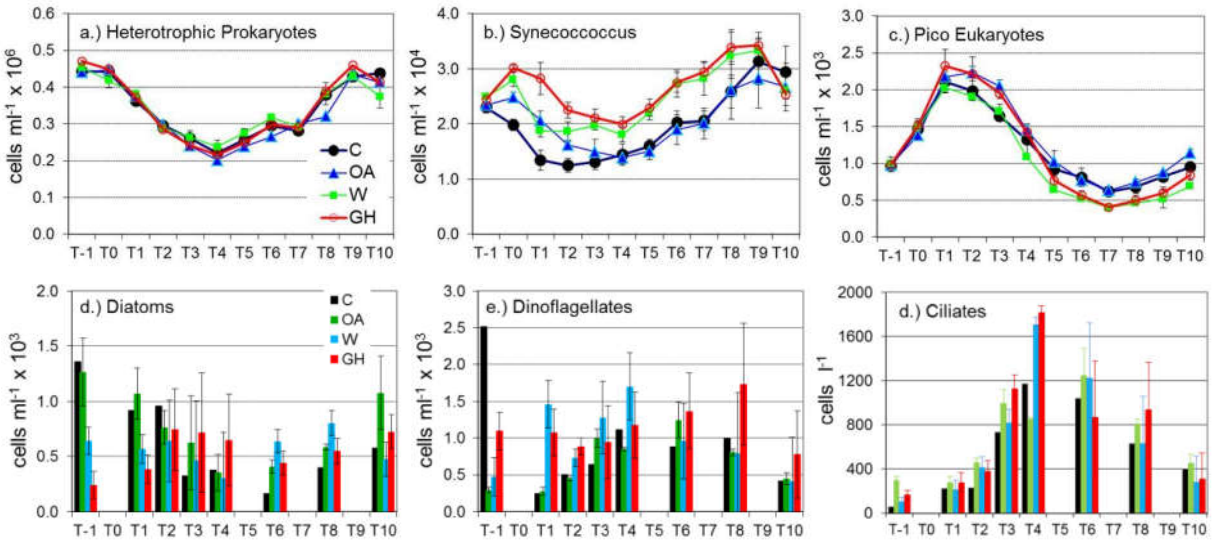

Fig. 2. Evolution of planktonic communities a.) Heterotrophic Prokaryotes b.) Synecococcus c.) Autotrophic Picoeukaryotes d.) Diatoms e.) Dinoflagellates and f.) Ciliates during the experiment (mean±sd of the 3 replicate mesocosms of each treatment). C: Control, OA: Ocean Acidification, W: Warming, GH: Greenhouse.

No direct effects of acidification on diatom, dinoflagellate and ciliate abundance was observed. Interestingly, a negative effect of increased temperature was observed on diatom concentration during the first days of the experiment, whereas on the other hand warming seems to favor dinoflagellate population (Fig. 2). The peak magnitude of ciliates recorded on day 5 in all mesocosms was more pronounced under warm conditions. The highest values were found in W and GH mesocosms (1800 cells l^{-1}).

Species-specific response to acidification and temperature increase may contribute to the dominance of different species. Elevated pCO_2 showed a more important increase of *Cylindrotheca* spp in diatom- assemblage composition during the two last days of the experiment (data not shown).

4. Discussion/Conclusions

Our data suggested a more rapid exploitation of elevated pCO_2 by photosynthetic picoplankton. Autotrophic cyanobacteria biomass profited from higher temperature/acidification despite limited nutrients. Elevated pCO_2 is known to affect autotrophic processes directly (Riebesell & Tortell, 2011), mainly picoeukaryotic photoautotrophs (Brussaard et al. 2013). The pCO_2 effects on bacterial production are rather indirect and in combination with other factors, influenced bacterial production throughout the experiment. Such effects can be related to increased phytoplankton biomass leading to a dissolved organic carbon (DOC) pool highly labile and rapidly taken up by heterotrophic bacteria. As reported by Malinsky-Rushansky & Legrand (1996) the percentage of extracellular release of dissolved organic compounds was found to be lower for larger algal cells than for picoeukaryotes.

The discrepancy between higher growth rates of bacteria in the GH, W mesocosms but similar standing stocks during the experiment might be attributed to a more active microbial food web-mediated carbon flow; higher viral lysis rates or grazing losses may limit biomass accumulation. The close coupling between production and grazing results in high rates of nutrient remineralisation

(Glibert, 1982). In the absence of external nutrient inputs primary production in the system is mainly fuelled by such "regenerated" nutrients. However increased remineralization of organic matter in such an oligotrophic environment can result in a reduced carbon export.

It is likely that different phytoplankton taxa react differently to ocean acidification. Mixed results on phytoplankton assemblages (Nielsen et al. 2012) have been reported and elevated pCO_2 shows no consistent effect on microzooplankton community composition (Suffrian et al. 2008). A thorough analysis of our microscope data on species composition is expected to provide additional knowledge on how ocean acidification and/or temperature increase has the potential to affect planktonic community composition and drive shifts in dominant species.

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