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Mesocosm CO_2 perturbation studies: from organism to community level

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1 Introduction

The uptake of anthropogenic CO_2 by the ocean and the corresponding increase in surface ocean CO_2 concentrations have already caused a measurable decrease in seawater pH. Surface ocean acidification through this process will amplify as long as fossil fuel CO_2 continues to enter the atmosphere and will transform the ocean to a new chemical state for tens of thousands of years. While the magnitude of these changes can be estimated with reasonable certainty for any given CO_2 emissions scenario, our understanding of their biological consequences is in its infancy. Effects of seawater acidification at the organismal level have been demonstrated in single species experiments and small-scale incubations of mixed assemblages. Among these, studies on plankton organisms have primarily focussed on coccolithophores (Riebesell et al., 2000; Zondervan et al., 2001, 2002; Sciandra et al., 2003; Leonardos and Geider, 2005; Langer et al., 2006), diatoms (Burkhardt et al., 2001; Rost et al., 2003), dinoflagellates (Rost et al., 2006), the diazotrophic cyanobacterium *Trichodesmium* (Barcelos e Ramos et al., 2007; Hutchins et al., 2007), foraminifera (Bijma et al., 2002), copepods (Kurihara et al., 2004), and sea urchin larvae (Kurihara and Shirayama, 2004). These studies have shown both adverse effects, including those on calcium carbonate production in calcifying organisms, and stimulating effects, such as on carbon and nitrogen fixation rates of some of the photoautotrophic organisms. In incubation experiments using mixed phytoplankton assemblages a shift in species composition from *Phaeocystis* to diatom dominance was observed with increasing pCO_2 (Tortell et al., 2002).

Through a variety of competitive and synergistic trophic interactions, the observed responses at the organism and population level can be transferred to the community and ecosystem level. Depending on the prevalence of negative and positive feedback loops, initial effects may be dampened or amplified, leading to gradual or catastrophic changes ("regime shifts") in community structure and functioning. Thus, for an integrated understanding of marine ecosystem responses to global change, there is a particular need for manipulative experiments on the whole community level. This can be achieved both in large enclosures and open ocean in situ experiments. While mesoscale in situ experiments, like the iron and phosphate fertilization studies, provide the best representation of whole ecosystems, logistically they are not always practical or feasible for manipulations other than iron enrichment. Here, mesocosm perturbation studies offer a reasonable alternative, allowing the manipulation of complex ecosystems under close to natural conditions in a range of oceanographic settings. Mesocosms also have the advantage of allowing different treatments in factorial or gradient design as well as the use of replicates, alleviating some of the statistical problems associated with in situ experiments (e.g. Thingstad et al., 2005, 2006).

Mesocosm manipulation experiments were successfully employed in recent studies examining the effects of changes in sea surface temperature (Sommer et al., 2007), mixed layer depth (Berger et al., 2006) and seawater pH/CO_2 (Kim et al., 2006) on pelagic systems. The effects of CO_2-induced seawater acidification on plankton communities were also addressed in a series of 3 mesocosm experiments, called the Pelagic Ecosystem CO_2 Enrichment (PeECE I-III) studies, which were conducted in the Large-Scale Mesocosm Facilities of the University of Bergen, Norway in 2001, 2003 and 2005, respectively (Fig. 1). Each experiment consisted of 9 mesocosms, in which CO_2 was manipulated to initial concentrations of 190, 350 and 750 μatm in 2001 and 2003, and 350, 700 and 1050 μatm in 2005 (Fig. 2; for further details see Engel et al., 2005; Grossart et al., 2006; Schulz et al., 2007). Results of the first two experiments are summarized in papers by Rochelle-Newall et al. (2004), Engel et al. (2003, 2005), Delille et al. (2005), Grossart et al. (2006), Benthien et al. (2007). This volume of Biogeosciences reports mainly on the results of the PeECE III experiment which was conducted between 15 May and 9 June 2005 and involved over 50 scientists from 14 European and North American institutions of which more than 30 scientists worked on site at the Espegrend Marine Biological Station (Fig. 3).

2 PeECE objectives

In line with the previous two experiments, PeECE III was set out to

1. test the validity of laboratory-based observations of CO_2/pH sensitivities in the natural environment
2. examine the transfer of such CO_2 sensitivities from the organism to the community level
3. assess their impacts on marine biogeochemical processes and air-sea gas exchange.

The PeECE I-III experiments not only allowed to study acidification effects on a complex, close to natural plankton community, they also provided the unique opportunity to bring together scientists from a wide range of disciplines, extending from molecular biology, marine microbiology and ecophysiology, biological oceanography, biogeochemistry, to marine and atmospheric chemistry.

3 Major findings

Although differences existed between experiments in some of the basic parameters, such as nutrient concentrations and stoichiometry, plankton species compositions and abundances, autotrophic and heterotrophic productivity, there was a surprising consistency in the overall robustness of the plankton communities to the applied CO_2 perturbations. The observed biological responses were largely dominated by the nutrient pulses added at the start of the experiment. As described by Tanaka et al. (this issue) for the PeECE III experiment, five phases can be distinguished during the course of the plankton development (Fig. 4): phase I – the initial period when all nutrients were replete, lasting until silicate was the first nutrient to become exhausted (day 6);

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phase II – extending until phosphate depletion (day 11); phase III – terminated by levelling off of nitrate drawdown (day 15); phase IV – characterized by more or less stable concentrations of all inorganic nutrients close to exhaustion levels with limited nutrient regeneration (day 20), phase V – marked by increased nutrient turnover.

It can not be ruled out that the pervasive response of the plankton community to the nutrient addition has masked possible effects caused by the CO_2 perturbations. In fact, no significant differences between CO_2 treatments were observed for

PeECE II+III

- concentrations of POM and DOM (Engel et al., 2004; Rochelle-Newall et al., 2004; Riebesell et al., 2007)

PeECE III

- phytoplankton composition and cell cycle during bloom development (Paulino et al., 2007)
- inorganic nutrient utilization, nutrient stoichiometry (Schulz et al., 2007; Bellerby et al., 2007; Løvdal et al., 2007) and nutrient turnover (Tanaka et al., 2007)
- biogenic calcification (Bellerby et al., 2007)
- bacterial abundance, diversity of attached bacteria, ^{14}C-leucine based bacterial production, bacteria-phytoplankton coupling (Allgaier et al., 2008)
- micro-zooplankton grazing (Suffrian et al., 2008)
- copepod feeding and egg production (Carotenuto et al., 2007)

In contrast, distinct CO_2 treatment effects were observed for

PeECE I

- biogenic calcification and carbon loss (Delille et al., 2005)
- stoichiometry of carbon to nutrient uptake and organic matter production (Engel et al., 2005)

PeECE II

- bacterial production and ectoenzymatic activities (Grossart et al., 2006)

PeECE III

- carbon drawdown, C:N:P stoichiometry of community production and carbon loss (Riebesell et al., 2007; Bellerby et al., 2007)
- cumulative ^{14}C primary production (Egge et al., 2007)
- diversity of free bacteria (Allgaier et al., 2008)
- viral abundance and diversity (Larsen et al., 2007)
- copepod nauplii recruitment (Carotenuto et al., 2007)
- DMS/DMSP concentrations (Vogt et al., this issue; Wingenter et al., 2007)
- chloriodomethane production (Wingenter et al., 2007)
- iron availability (Breitbarth et al., personal communication)

A thorough interpretation of CO_2/pH sensitivities observed for some components and processes of the pelagic system and of their apparent absence for others requires a careful consideration of time scales. From a methodological perspective relevant time scales include i) the rate and magnitude of the initial CO_2/pH perturbation, e.g. in relation to corresponding natural variations and to the projected rate of future environmental change, ii) the duration of the experimental period, e.g. in relation to the duration of the specific event covered by the experiment (in this case a plankton bloom) as well as the generation time of the organisms involved. From the biotic perspective, relevant time scales are those of bio-acclimation and adaptation as well as for the transfer of responses from the organism to the community and ecosystem level. Based on these considerations, attempts should be made to distinguish between stress-related responses and sensitivities expressed under full acclimation as well as between acute and chronic effects.

The same critical assessment should also be applied when interpreting the absence of perturbation responses, particularly with regard to secondary effects. For instance, is the time scale of observation sufficient to allow for a response at one trophic level to be effective at another level? This will help to assess whether or not the absence of a response can be regarded as true evidence for non-sensitivity.

With our present level of understanding of the pelagic food web, generalizations from single mesocosm experiments require caution. Simple models suggest that the system may have different states, with corresponding differences in behaviour. One relevant example would be the possibility of bacterial growth being limited by either mineral nutrients or organic carbon (Thingstad et al., 1997), where it is quite conceivable that indirect effects may propagate to the bacterial level in different manners depending on the state of the system. An indication for this is in fact provided by the observed differences in CO_2/pH sensitivities of bacterial production between PeECE II (Grossart et al., 2006) and PeECE III (Allgaier et al., 2008).

4 Future directions and challenges

An integrated assessment of the effects of global change on marine ecosystems and biogeochemical cycling requires a combination of i) process studies of contemporary forcing in the field, ii) manipulative experiments examining the responses of the marine biota to projected future forcing, and iii) coupled biogeochemical ecosystem modelling. Applying these three approaches in an interactive manner is required to achieve realistic projections of future ocean change.

Manipulative experiments can be executed on various scales ranging from well-controlled laboratory assays to whole ecosystem perturbation studies. While the underlying biochemical and physiological mechanisms involved in organism responses can generally be best studied in well-controlled laboratory experiments, understanding the transfer of these responses to the community and ecosystem level requires larger scale community level experiments. Recent mesocosm experiments have provided a wealth of information on the sensitivities of natural assemblages to ocean change. They have also highlighted again certain limitations in mesocosm approaches which call for careful examination of the available data sets, inter-comparison of different mesocosm experiments and further development of the mesocosm approach. Some of the challenges for future mesocosm experimentations will be:

- Avoiding perturbations other than the one to be tested. This includes unintended perturbations, for instance due to nutrient addition or strong agitation of the enclosed water during filling of the mesocosms (e.g. by means of pumps or through artificial mixing of the enclosed water column). It should be noted here that CO_2 aeration itself creates a considerable perturbation, which can lead to flocculation of dissolved organic matter. This was observed to greatly stimulate bacterial production during the starting phase of the PeECE III experiment. Also, enclosing a volume of water represents a perturbation in itself. Hence, while it is instructive to compare the development inside the mesocosms with that in the ambient water, the ambient should not be seen as control for the enclosed water.

- Prolonging the duration of mesocosm experiments to cover periods prone to acclimation and possibly adaptation processes. With increasing evidence now suggesting micro-evolutionary adaptation to be a potentially important dampening mechanism in response to global change, this should be a top priority of future research in global change biology. Longer experiments may also be needed to cover the life cycles of sensitive key species, including most critical phases such as egg and larval development. Obviously, there is a trade-off in prolonging the experimental period due to the increasing importance of wall effects and other enclosure related side effects (e.g. on turbulence and water column mixing) leading to an increasing deviation from the natural system with time.

– Extending mesocosm application beyond in-shore systems to allow the study of open water key ecosystems and biogeochemical provinces. To provide more flexibility in the selection of ecosystem types and oceanographic setting, a mobile mesocosm facility is presently being developed as part of the German SOLAS Programme SOPRAN (Surface Ocean Processes in the Anthropocene). A first off-shore mesocosm experiment employing 6 free-floating mesocosms each enclosing 65 m^3 of water has been conducted in the Baltic proper during July of 2007. Key study areas identified for future off-shore mesocosm experiments are the high latitude polar seas, high productivity systems in temperate zone, and subtropical systems dominated by diazotrophic cyanobacterial communities.

– Increasing the volume of mesocosm enclosures to allow for the inclusion of higher trophic levels, including micronekton. Considering limited financial resources, this may be at the expense of replication, shifting from multiple medium-sized to large-scale, single treatment and control enclosures.

– To ensure comparability of the results from mesocosm experiments it will be important to develop guidelines and quality standards for best practice. This should include questions concerning extrapolation of mesocosm results to the natural system, optimal mesocosm size for the specific community to be examined, closed versus open systems, and replication and controls. To promote comparative studies on results from multiple mesocosm experiments it will also be extremely helpful to collect and archive the data centrally and make them available to the scientific community.

It is worth noting here that there is a certain attraction in conducting in situ CO_2 perturbation experiments at the scale of previous iron fertilisation experiments, in an attempt of avoiding short-comings associated with mesocosm enclosures. However, aside from the complications also encountered with in situ iron fertilization experiments, such as (1) lateral dilution of the fertilised patch, (2) lack of replication and (3) vertical and horizontal migration of micronekton in and out of the patch, a meso-scale in situ CO_2 perturbation is logistically extremely demanding. Acidifying a patch of 10×10 km in size and 50 m depth from pH 8.1 to pH 7.8 requires approximately 30 000 tons of CO_2 or 54 000 tons of concentrated HCl, i.e. beyond the capacity of conventional research ships. Moreover, as the effects of ocean acidification on the marine biota are likely to scale with the degree of CO_2/pH change, a gradient of multiple CO_2 levels in enclosures of intermediate size appears to be more appropriate than a single large-scale in situ perturbation experiment. A CO_2 gradient approach will also be better suited for the assessment of critical threshold levels beyond which irreversibly changes may occur.

5 Summary

Mesocosm studies have provided and continue to provide a wealth of information on pelagic ecosystem responses to CO_2 induced changes in seawater chemistry (Engel et al., 2004, 2005; Delille et al., 2005; Grossart et al., 2006, PeECE[1]). The suitability of this technique for conducting interdisciplinary research combining marine ecosystem and biogeochemical approaches with aspects relevant to marine and atmospheric chemistry has been successfully demonstrated. The set of CO_2 perturbation experiments conducted until now provides a comprehensive but complex data set which lends itself for detailed meta-analyses to further explore the interplay between the dominant ecosystem drivers and to determine which processes are important to be incorporated in marine ecosystem and biogeochemical models. In spite of some limitations, in situ

[1]PeECE: Pelagic Ecosystem CO_2 Enrichment Studies, Special Issue, Biogeosciences

mesocosm perturbation studies provide an effective tool to unravel the effects of projected future forcing on natural aquatic ecosystems and will provide the link between in vitro experiments and field observations. As human-induced global change continues to alter marine environmental conditions, manipulative experiments at the community to whole ecosystem level will become increasingly relevant.

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Fig. 1. PeECE III experimental set-up at Large-Scale Mesocosm Facility of the University of Bergen in Espegrend, Norway. Left: array of 9 mesocosms in front of the floating raft. Right: Mesocosm enclosures were covered by gas-tight tents made of ETFE (ethylene tetrafluoroethylene) foil, which allowed for 95% light transmission of the complete spectrum of sunlight, including UVA and UVB.

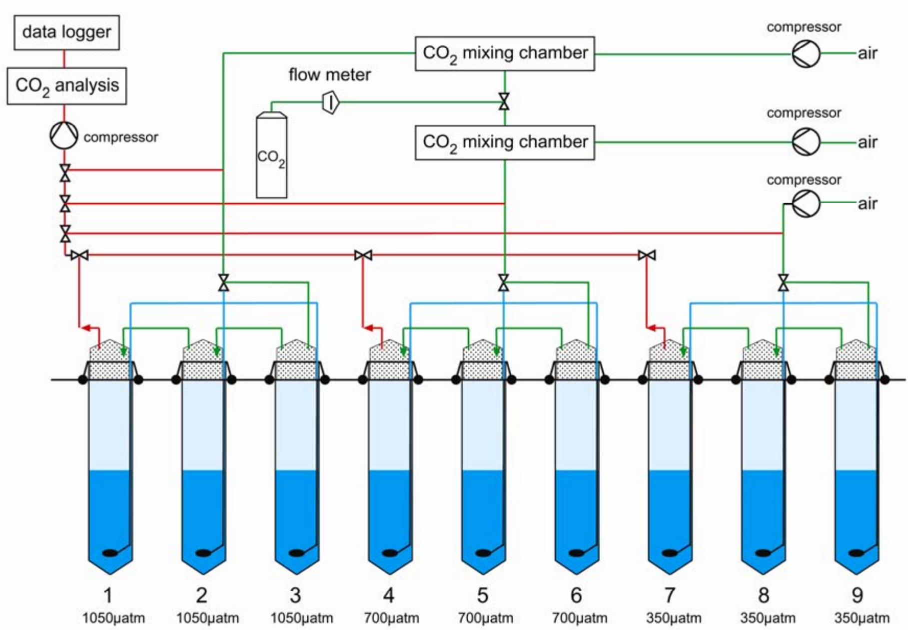

Fig. 2. Sketch of experimental set-up: Green lines indicate supply of air and CO_2-enriched air into the headspace (continuously aerated throughout the experiment); blue lines show the corresponding supply into the water column (aeration of the water column started 3 days prior to the beginning of the experiment and was simultaneously discontinued in all mesocosms when target pCO_2 levels were achieved; day 0 of the experiment). Red lines mark intake for continuous pCO_2 measurements. Light and dark blue shading indicate separation of the water column into upper mixed and bottom layers maintained by a salinity offset of 1.5 psu at 5.5 m water depth (established by addition of freshwater into the mixed surface layer after terminating aeration of the water column water). Bottom line gives pCO_2 values maintained in the headspace and for each mesocosm.

Fig. 3. On site participants of PeECE III study at the Espegrend Marine Biological Station: *Left to right: first row:* Paolo Simonelli, Ylenia Carotenuto, Kerstin Suffrian, Julia Wohlers, Aurelie Colomb, Haimanti Biswas, Ruth–Anne Sandaa, Evy Foss Skjoldal, Aud Larsen, Peter Fritsche, Noureddine Yassaa, Christian Schlosser; *second row:* Tsuneo Tanaka, Jens Larsen, Eckart Zöllner, Marius Müller, Joana Barcelos e Ramos, Martin Allgaier, Ana Paulino, Michael Meyerhöfer, Jorun Egge, Vianyak Sinha; *third row*: Karl Haase, Sebastien Putzeys, Ulf Riebesell, Kai Schulz, Mikal Heldal, Jens Nejstgaard, Eike Breitbarth, Craig Neill, Jonathan Williams. PeECE III participants not in this picture: Avan Antia, Jørgen Bendtsen, Richard Bellerby, Gunnar Bratbak, Lei Chou, Marion Gehlen, Hans-Peter Grossart, Rolf Hofmann, Truls Johannessen, Thomas Klüpfel , Veronique Martin, Jack Middelburg, Dirk Neumann, Torkel Gissel Nielsen, Gisle Nondal, Nils Arne Sæbø, Philippe Saugier, Birgit Søborg, Karoline Soetart, Runar Thyrhaug, Susan Turner, Michael Steinke, Frede Thingstad, Meike Vogt, Oliver Wingenter, Max Ziegler.

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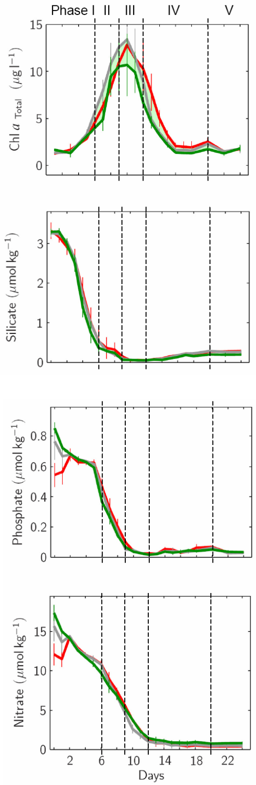

Fig. 4. Development of the plankton community during the experiment: Based on nutrient availability and turnover, 5 phases can be distinguished: phase I – start of the experiment until the onset of silicate limitation (day 6), all nutrients replete; phase II – nitrate and phosphate replete, terminated by phosphate limitation (day 9); phase III – ends with onset of nitrate limitation (day 12); phase IV – characterized by more or less stable concentrations of all inorganic nutrients close to exhaustion levels with limited nutrient regeneration (day 20), phase V – marked by increased nutrient turnover (see Tanaka et al., 2007).