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Loss of Mediterranean marine biodiversity in a high-CO2 world

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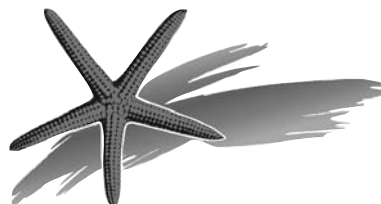
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**Impacts of acidification
on biological, chemical and physical systems
in the Mediterranean and Black Seas**

Menton, 1 – 4 October 2008

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This collection offers a broad range of titles in the marine sciences, with a particular focus on emerging issues. The Monographs do not aim to present state-of-the-art reviews; they reflect the latest thinking of researchers gathered at CIESM invitation to assess existing knowledge, confront their hypotheses and perspectives, and to identify the most interesting paths for future action.

A collection founded and edited by Frédéric Briand.
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I - EXECUTIVE SUMMARY OF CIESM WORKSHOP 36

“Impacts of acidification on biological, chemical and physical systems in the Mediterranean and Black Seas”

by

Yilmaz A., De Lange G., Dupont S., Fine M., Fowler S.W., Gattuso J.P., Gazeau F., Gehlen M., Goyet C., Jeffrey R., Montagna P., Rees A.P., Reynaud S., Rodolfo-Metalpa R., Ziveri P. and F. Briand

This synthesis was drafted under the coordination of Aysen Yilmaz by all workshop participants, with special thanks to Scott Fowler and Marion Gehlen. The Monograph Series Editor, Frédéric Briand, reviewed and edited the entire volume, assisted by Valérie Gollino for the physical production process.

1. INTRODUCTION

The Workshop took place from 1 to 4 October 2008 at the Hotel Royal Westminster in Menton, within walking distance of the border separating France from Italy. Seventeen researchers from ten countries (see list at the end of this volume) attended this exploratory meeting at the invitation of CIESM. Frédéric Briand, Director General of the Commission, did warmly welcome the participants, reminding them that the workshop had been scheduled deliberately on the eve of the 2nd SCOR International Conference on 'Ocean in a high CO₂ World' - due to open in Monaco on the following week - so as to draw attention to this issue for the Mediterranean Sea, a region where the impact of acidification is still hardly known. A challenge for the participants would be to draw a much needed inventory of major gaps in, and priorities for, acidification research within the specific regional context. Professor Briand then introduced Professor Aysen Yilmaz, Chair of the Committee on Marine Biogeochemistry, who presented an overview of the scientific background and key objectives of the meeting, which would seek to take optimal advantage of the original mix of chemical and biological expertise brought along by the invited participants.

As a result of human activity, the chemistry and biology of the marine world are fast changing. This is particularly notable in terms of carbon emissions: since the beginning of industrialization, between 1800 and 1999, mankind has emitted 361 Gt C to the atmosphere of which the ocean has absorbed approximately 155 Gt C (Sabine *et al.*, 2004). This makes the world ocean the largest sink of anthropogenic CO₂.

The uptake of CO₂ by the ocean is primarily a physico-chemical process. As CO₂ penetrates seawater, it behaves like a weak acid dissociating according to:



which leads to an increase of [H⁺] and thus a drop in pH. This process, now well documented, results in acidification of marine waters on a global scale. It is referred to as ‘Ocean Acidification (OA)’ (Caldeira and Wickett, 2003; Orr *et al.*, 2005).

Acidification of ocean water occurs in tandem with a decrease in carbonate ion concentration and the saturation state with respect to carbonate minerals (CaCO₃), which directly impacts its formation and dissolution. In the marine environment, carbonate formation is largely a biotic process (Milliman, 1993). Marine organisms form shells and skeletons composed of a variety of carbonate minerals: high-Mg calcite (coralline algae), aragonite (pteropods and corals, some foraminifera and bivalves), calcite (coccolithophores, foraminifera, some bivalves), as well as mixed-layered calcite and aragonite (certain bivalves). The solubility of a carbonate mineral depends on its composition and structure; it increases in the order of calcite, aragonite and high-Mg calcite. One can easily envision that these differences in solubility translate into variable susceptibility to dissolution of these minerals. Likewise one can also expect differing degrees of vulnerability of calcifying organisms to ocean acidification. In addition to changes in the carbonate system, OA is expected to influence ocean chemistry, affecting the availability and speciation of nutrients and toxins to marine organisms.

The general impact of OA on water chemistry is well understood but regional data and models are needed. An even more urgent need is to assess the impact on ecosystems. Study of the impact of OA at the biological level is in its infancy and there is little known about present and future impacts on marine organisms and ecosystems. This is particularly true for the Mediterranean and the Black Seas since these mid-latitude, semi-enclosed water bodies are not projected to experience the first effects of acidification such as occur in high latitude seas. The scant available data reveal contradictory results (increased and decreased calcification) (e.g. coccolithophores, Ziveri *et al.*, this volume) and apparent paradoxes (e.g. corals, Fine and Mordechay, this volume; early life history, Dupont and Thorndyke, this volume). Furthermore the eastern and western basins of the Mediterranean Sea are significantly different environments, both chemically and biologically, so that the potential effects of OA on their chemistry and biology could substantially differ. At this early stage, based on limited present knowledge, it would be risky to extrapolate from one species to another and from one region to another. There is clearly an urgent need to predict the near future consequences of OA in Mediterranean and Black Sea species and ecosystems and to determine what species/ecosystems are most at risk.

Given the potential of OA to appreciably affect marine biodiversity, it is important as well to make initial evaluations of the possible negative socio-economic impacts, related for example to the fisheries and tourism industries.

This report presents the outcome of our discussions on where there are gaps in our knowledge, which data are needed urgently so as to predict potential impacts of OA on marine ecosystems in the near future. Based on available data, we will define priorities and suggest guidelines for future work.

2. IMPACTS OF HIGH *p*CO₂ ON MEDITERRANEAN AND BLACK SEA CHEMISTRY

2.1. Impacts of high temperature and *p*CO₂ on the chemistry of the Mediterranean and the Black Seas: emphasis on carbon cycle, carbonate chemistry, aragonite and calcite saturation horizons, and on pH

As the seawater temperature rises, we expect a slight decrease in the air-sea CO₂ fluxes due to the change in CO₂ solubility. On the other hand, as CO₂ rises in the atmosphere, we can expect a slight increase in the air-sea CO₂ fluxes due to the larger difference in *p*CO₂ between the air and the surface seawater. Consequently, the increase or decrease of CO₂ penetration into the Mediterranean and Black Seas will strongly depend upon the rate of CO₂ rise in the atmosphere. However since the temporal response of the CO₂ air-sea flux is relatively slow (one year), the CO₂ increase in the

atmosphere would probably be more rapid than in the sea-surface and will mitigate the temperature effect, which would tend to decrease the penetration of anthropogenic carbon in seawater.

If the chemistry of the CO₂/carbonate system in seawater is well known, the amplitudes of variation of temperature and anthropogenic CO₂ penetration that will influence the chemical equilibrium are not. For instance, it is known that as anthropogenic CO₂ penetrates into the surface water by air-sea gas exchange, it raises both the concentration of total CO₂ (C_T), and the partial pressure of CO₂ (pCO₂). As pCO₂ in sea-water increases, CO₂ fluxes from the atmosphere into the sea surface decrease. In addition, pCO₂ is very sensitive to temperature increase (~ 4,3 % per °C). Thus, both the increase in sea-surface temperature and the penetration of anthropogenic carbon into the sea-surface will increase the sea-surface pCO₂, reducing the penetration of anthropogenic carbon into seawater. As the Mediterranean Sea is relatively more alkaline than the open ocean, during a given time-period it can absorb relatively more anthropogenic CO₂ than the open ocean. In other words its potential pH change (decrease) is relatively large compared to that of the Atlantic Ocean. As Mediterranean seawater is warmer (> 13°C) and more alkaline than the open ocean throughout the water column (Touratier and Goyet, in press), its saturation state (Ω) with respect to calcium carbonate will remain saturated throughout most of the water column for many years to come.

Total alkalinity (A_T) concentrations measured in the Mediterranean Sea are high (2,600 μmol kg⁻¹), which was attributed to evaporation coupled with high freshwater A_T inputs into coastal areas (Schneider *et al.*, 2007). Mediterranean waters are supersaturated with respect to calcite and aragonite; the excess alkalinity likely reflects alkalinity inputs to coastal areas close to regions of deep and intermediate water formation. An alkalinity budget shows that the main alkalinity inputs come from the Black Sea and from rivers, whereas the Strait of Gibraltar is a net sink (Schneider *et al.*, 2007). The major sink appears to be carbonate sedimentation. The basin-average net calcification rate and CaCO₃ sedimentation was estimated to be 0.38 mol m⁻² yr⁻¹. The estimated residence time of A_T is 160 yr (Schneider *et al.*, 2007).

In the late 1980s and early 1990s, abrupt changes in salinity and in temperature caused a continuous increase of density and massive deep water formation in the south Aegean Sea that altered the thermohaline circulation of the eastern Mediterranean with consequences also in the distribution of other environmental parameters (Roether *et al.*, 1996; 2007; Malanotte-Rizzoli *et al.*, 1999; Klein *et al.*, 1999). Deep water formation areas in the Mediterranean may be responsible for setting the air-sea CO₂ balance and act to sequester CO₂ and increase the sink capacity in such areas.

There are less data on the carbonate chemistry of the Black Sea (Tyrrell *et al.*, 2008). Goyet *et al.* (1991) measured total inorganic carbon (C_T) and total alkalinity (A_T), plus other carbonate system parameters derived from them at a single station in 1988. Surface water concentration of carbonate ion was 250 μmol kg⁻¹, and calcite saturation state was 7.5, that is, similar to the data collected in the central Black Sea in 2001 by Hiscock and Millero (2006). Data on the present calcium carbonate saturation state for the Black Sea surface waters are limited, but appear to remain strongly supersaturated year-round.

2.2. Impacts of acidification on biogeochemical cycling: emphasis on nutrients, trace elements and primary production

Robust information on the flux of nutrients and biogeochemical cycles under the influence of enhanced pCO₂ is scarce at global level, and appears to be non-existent for the Mediterranean. Following a review of published information and a number of small scale experiments, we tentatively propose that ocean acidification will act to increase the oligotrophic nature of the Mediterranean Sea and increase the degree of phosphorus limitation currently found which, almost by definition, will contribute to reduced productivity and carbon export.

Theoretical considerations of nutrient equilibria with pH (Zeebe and Wolf-Gladrow, 2001) would suggest significant reductions in PO₄ concentration and alteration of the NH₄ ⇌ NH₃ equilibrium at a pH decrease of 0.3, although two independent studies in northern waters have recorded little or no change in both PO₄ and NO₃ under this altered pH regime (Tanaka *et al.*, 2007; Rees *et al.*, this volume).

Two studies in Pacific (Hueseman *et al.*, 2002) and Atlantic coastal waters (Rees *et al.*, this volume) have indicated that nitrifying bacteria may be sensitive to OA. Reductions in nitrification rate of 20 – 30% have been suggested, which may have implications for the balance of new to regenerated productivity in surface waters and in the balance of the nitrification: denitrification couple in sediments and anoxic waters. Changes in the ammonium:nitrite:nitrate ratios may alter microbial species activity and diversity, with consequences on ecosystem function and the release of nitrous oxide.

Denitrification and sediment nutrient fluxes are also liable to be impacted by OA (Widdicombe and Needham, 2007; Rees *et al.*, this volume). This has the potential to change microbial activity and both the type of bioturbatory organisms present and the intensity of their activities in the transport of nutrients across the sediment-water interface. There is evidence to suggest that under current reduction scenarios for ocean pH, NO₃ removal from, and NH₄ release to, overlying waters may be enhanced. This would further alter the NO₃:NH₄ stoichiometry, with implications for ecosystem function and algal community composition.

Recent laboratory studies with cultured *Trichodesmium* have shown that N- and C-fixation rates increased significantly with elevated CO₂ (Hutchins *et al.*, 2007; Levitan *et al.*, 2007; Barcelos e Ramos *et al.*, 2007). Particularly relevant to the P-impooverished Mediterranean is the study of Hutchins *et al.* (2007) who found that higher CO₂ enhanced nitrogen fixation and growth rates even under severely phosphorus-limited steady-state growth conditions. However, our knowledge of the distribution and activity of diazotrophs under natural conditions in the Mediterranean is limited, and the dangers of extrapolating from a single species to the level of community effects need to be considered.

Decreases in ocean pH generally increase the proportion of free dissolved trace metals (Royal Society, 2005), thereby increasing metal solubility. This could in theory lead to substantial increases in the total bioavailable fraction of many trace elements, perhaps resulting in toxic concentrations. Micronutrients (e.g. Fe, Co, Zn, Cd and Cu) are essential components of numerous metalloenzymes, which ultimately regulate microbial activity, productivity and thus carbon export in the oceans. The variable sporadic supply of atmospheric Saharan dust is considered perhaps the most significant source of many essential metal nutrients such as Fe to the eastern Mediterranean (Statham and Hart, 2005). The present view is that Fe is highly unlikely to be limiting in the Mediterranean basins (Ozsoy and Saydam, 2001; Statham and Hart, 2005). Thus changes or increases in the bioavailable fraction of many trace metals due to ocean acidification of the Mediterranean are more likely to result in changes in the composition of microbial assemblages and/or toxic effects. Increased local regional variability in the manifestation and magnitude of these changes will be of paramount importance, e.g. potentially to the fishing industry.

The response of primary producers to OA is currently contentious and should be given priority for research activity. Whilst there have been a number of reports of enhanced organic carbon fixation at elevated CO₂, the opposite has also been reported. At present coccolithophores are the main calcifying group in the Mediterranean and Black Seas. Published culture results show prevailing uncertainties regarding the controls on coccolithophore calcification response to anthropogenic OA. Adaptation to changes in carbonate ion concentration (e.g. basic building block of skeletons and shells for a large number of marine organisms) is also unknown. It is worth noting that the phytoplankton population of the modern eastern Mediterranean is dominated by picoplankton (Li *et al.*, 1993) and nanoflagellates, including coccolithophores (Ziveri *et al.*, 2000; Cros, 2001; Malinverno *et al.*, 2003).

The strong uptake of anthropogenic CO₂ by the Mediterranean Sea is significantly altering its seawater chemistry, particularly in the coastal zones. Acid-base imbalance and reduced oxygen transport capacity are some of the high CO₂ influences on the physiology of marine organisms (Fabry *et al.*, 2008; Widdicombe and Spicer, 2008). Today, one of the main limits to quantify and predict the impacts of ocean acidification is the scarcity of temporal observations of CO₂ penetration in seawater. The first results of such observations in the Mediterranean Sea are from the DYFAMED time-series station. The main results (Touratier and Goyet, in press) indicate that:

- 1) The concentrations of anthropogenic CO₂ in the Mediterranean are much higher than those found in the Atlantic Ocean (the minimum concentration at the DYFAMED site is ~50

$\mu\text{mol.kg}^{-1}$). Consequently, the Mediterranean seawater already presents significant pH drops. Furthermore, the Mediterranean Sea is a source of anthropogenic CO_2 for the Atlantic Ocean.

2) Anthropogenic CO_2 is decreasing with time, especially in the intermediate and the deep layers in the water column at the DYFAMED site. This decrease of anthropogenic CO_2 in deep waters is significantly correlated with a decrease in the dissolved oxygen and with an increase in both salinity and temperature. The decrease of anthropogenic CO_2 resulted from an invasion of old water masses. In order to better understand the origin of this reduction, several hypotheses – such as increased stratification limiting the nutrient supply and high anthropogenic CO_2 penetration – are currently investigated.

2.3. How Mediterranean and Black Sea environmental conditions have changed due to changes in pH in the past: “Ocean Acidification” and lessons from history

To assess the impact of future effects of changing environmental conditions in the Mediterranean, we need to explore what lessons can be learned from the past. Subsequently this information can serve as a basis for validating model simulation studies on future impacts.

Abrupt climate changes are known to have occurred in the Mediterranean in the past, resulting in major changes not only in the accumulation of C_{org} but also in that of calcium carbonate. Assessments of ocean acidification usually refer to the Mediterranean as being supersaturated with respect to carbonate, thus not a potentially impacted basin. However, the short water mass ventilation time of ~ 100 years suggests a rapid potential response to changing environmental conditions. When looking into past records, rapid variations in the Mediterranean acidification state appear to have occurred. These are most clearly expressed during periods of formation of organic-rich units (sapropels), often coinciding with considerably reduced sedimentary carbonate contents. Such observations may be related to changes in ecosystem, surface water biogeochemical processes, calcification of planktonic calcifying organisms, and partly also to dissolution events. These variations must be related to the very subtle balance in the eastern Mediterranean where slight environmental/climatic disturbances have resulted in major and abrupt system perturbations with dramatic consequences (Figure 1; De Lange, unpublished data). Not only do we find such major changes on the ~ 20 kyr scale, distinct but, more minor variations also appear to have occurred at higher frequency. Such major perturbations do not seem to govern the Western Mediterranean in the same way. In the present and in the past as well, environmental conditions apparently were different between the eastern and western Mediterranean resulting in distinct ecological responses. These differences are at least partly related to differences in thermohaline circulation and to the overall circulation pattern, the latter being more restricted and more sensitive to changes related to environmental forcing. The present-day situation is forced by the increasingly rapid rise of anthropogenic carbon into the atmosphere (Goyet and Touratier, this volume), thus provoking numerous impacts on the physical, chemical and biological properties of the Mediterranean Sea. The fast increasing rate of these changes makes them now observable over only a few decades, making continual observations essential.

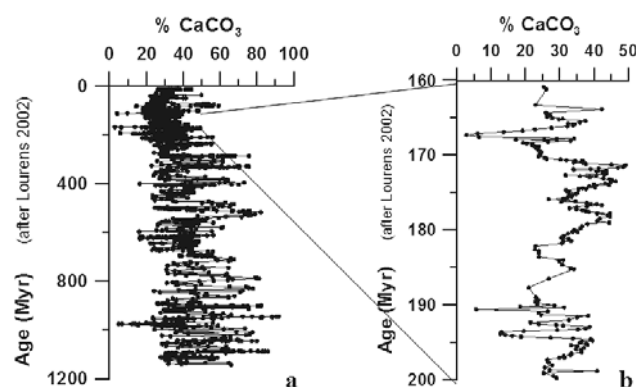


Figure 1. Illustration of major rapid changes in eastern Mediterranean sedimentary carbonate contents over (a) the last 1.2 million years, and (b) the 160-200 kyr time interval. Sample resolution is ~ 500 years. (De Lange, unpublished data).

No published data are yet available for the carbonate response of planktonic calcifying organisms to past changes in sea surface water carbonate chemistry in the Mediterranean (Ziveri *et al.*, this volume). The rate of calcium carbonate burial and the contribution of coccoliths to this amount can, however, be evaluated by estimates of species-specific coccolith weight (Young and Ziveri, 2000; Beaufort, 2005). The morphology and thickness of individual coccoliths help to evaluate past calcite production.

Deep- and intermediate water formation – and therefore ventilation – may have been much more variable in the eastern basin than in the western basin. Consequently, much larger perturbations are observed in the deep eastern Mediterranean sediment record. Such past perturbations have resulted in more acidic deep water characteristics, in the enhanced preservation of organic matter and dissolution of carbonate, and thus in enhanced sequestration of CO₂. One way to assess the differences in the past deep-water ventilation among the Mediterranean basins is through the investigation of the geochemical signals in natural marine archives, such as the deep-water corals inhabiting the intermediate and deep-waters. Cold-water corals are among the most promising archives for intermediate and bathyal water depths, generally providing higher resolution (although more discontinuous with time) than sediment cores (e.g. sub-decadal vs. 100-500 years for sediments), and not being affected by bioturbation. Moreover, cold-water corals can be radiometrically dated using both high precision U-series and ¹⁴C methods. By coupling radiocarbon data with P/Ca ratios – a technique which has been recently demonstrated to be a proxy for nutrients in azooxanthellate corals (Montagna *et al.*, 2006) – from the same absolutely dated sample (U/Th) it will be possible to calculate the ventilation rate for specific time-windows in the past. The P/Ca encoded in the skeletal aragonite provides information on the phosphate content of individual water masses in the past and represents a water mass tracer, giving constraints on the mixing ratio between distinct compositional end-members. Further, 4P/Ca micro-analyses (Montagna *et al.*, this volume) will allow us to quantify the fluxes of nutrients to intermediate and deepwater environments and assess the past ocean productivity, thereby furthering our understanding of the biological functions of the Mediterranean Sea in regulating atmospheric CO₂. Suggested future changes, i.e. in deep-water formation, thus in residence times for deep and intermediate water masses, will influence all of these properties.

3. MODELING STUDIES FOR BETTER UNDERSTANDING AND PREDICTIONS OF FUTURE IMPACTS

The Mediterranean basin is a potential hotspot for environmental change. Anthropogenic stressors such as increasing river nutrient loads, atmospheric inputs, pollution, fishing and aquaculture activities combine with climate change-induced temperature increase and changes in seawater chemistry. High alkalinity and high levels of super-saturation of surface waters are distinct features of the Mediterranean Sea (Schneider *et al.*, 2007). Taken together these characteristics have led to the conclusion that impacts of ocean acidification would be less severe in the Mediterranean Sea than in other oceanic regions (e.g. Arctic Ocean). While a growing number of studies address changes in seawater carbonate chemistry in response to rising atmospheric CO₂ at the global or regional scale, we are not aware of studies targeting the Mediterranean or Black Sea. It is tempting to turn towards state-of-the-art coupled climate carbon models for a first order evaluation of effects. However these models lack the appropriate spatial resolution and process representation for deriving a realistic description of the Mediterranean and Black Seas. A typical model output is shown here (Figure 2) depicting the saturation state of the upper 100 m of the water column with respect to aragonite (Ω_a) in the global ocean and the Mediterranean Sea respectively. Model results are from the French (IPSL) coupled climate carbon cycle model for a standard IPCC business as usual scenario (SRESA2). The model projects a decrease in surface ocean Ω_a from ± 4 in pre-industrial times to ± 2 at the end of the scenario. Therefore Mediterranean surface waters would remain oversaturated with respect to aragonite.

Studies of the calcification response of various organisms (Kleypas *et al.*, 2006 and references cited there) suggest a reduction of calcification rates before undersaturation is reached. Similarly, saturation states of ≤ 2 are referred to as marginal for tropical corals. Recent results (Iglesias-Rodriguez *et al.*, 2008) challenge the straightforward relationship between calcification and

saturation. The biological impacts of changes in carbonate chemistry projected by the model need to be addressed through studies targeting Mediterranean calcifying organisms, both benthic and pelagic.

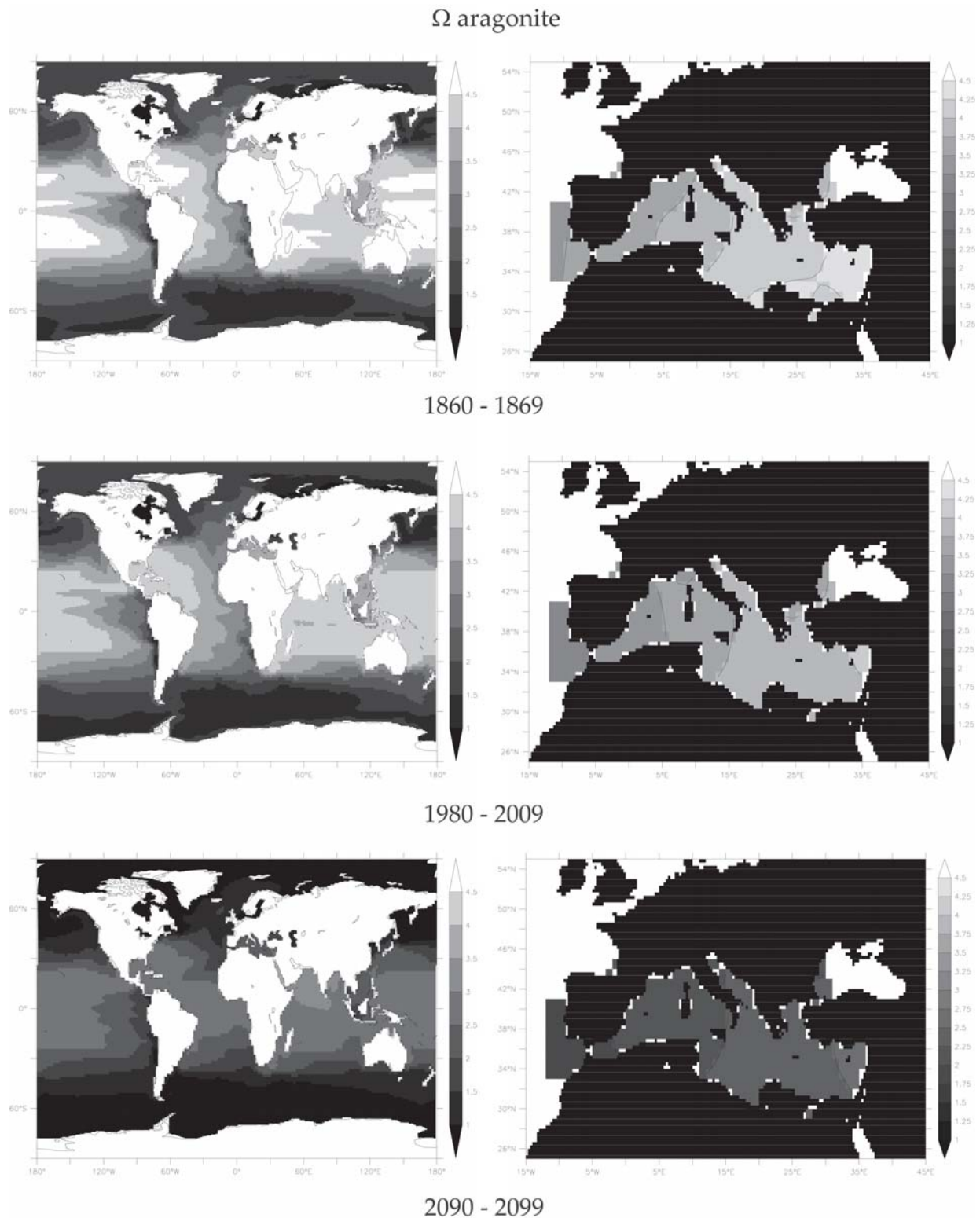


Figure 2. Saturation state with respect to aragonite averaged over top 100 m of water column in the global ocean (left panel) and Mediterranean Sea (right panel), computed for the preindustrial (top), present day (middle) and 21st century (bottom) by a coupled coarse resolution climate carbon cycle model.

While such a coarse resolution model allows a first assessment of changes in Mediterranean Sea carbonate chemistry in response to global change and rising atmospheric CO₂ levels, it does not resolve the complexity of Mediterranean hydrodynamics (e.g. rates and sites of deep water formation and variability). An improved representation of Mediterranean hydrodynamics is obtained from regional coupled atmosphere ocean models (Somot *et al.*, 2006; 2008), which predict a decrease in deep water formation and thus a slowing down of the Mediterranean thermohaline circulation in response to climate change. A slowing down of deep water formation will isolate intermediate and deep waters from exchange with the atmosphere. This event could have consequences for anthropogenic carbon sequestration and for deep water chemistry. Such impacts need to be quantified on a Mediterranean basin scale and across the Mediterranean Sea outflow into the North Atlantic.

4. BIOLOGICAL IMPACTS OF OCEAN ACIDIFICATION IN MEDITERRANEAN AND BLACK SEA ECOSYSTEMS

4.1. A rich biodiversity at risk

The Mediterranean maintains overall low biological productivity and a high species diversity with an estimated 8,560 species (between 4 - 18 % of all described marine species), many of which are endemic. This is primarily because it is a warm sea in temperate latitudes and therefore hosts both temperate and subtropical species. There is a notable east to west gradient in increasing species richness which closely reflects climate gradients. Over the last few decades a northward migration of certain species associated with warming waters has been demonstrated in several areas of the western Mediterranean (CIESM, 2008).

The number of species in the Black Sea is around one third of that in the Mediterranean. Despite recent changes in absolute numbers, the ratio remains close to 2.3, with approximately 8,500 species in the Mediterranean *versus* 3,700 in the Black Sea. There have been dramatic changes in species diversity, particularly in the northwestern portion of the shelf, caused by eutrophication and subsequent hypoxia and anoxia conditions observed in the late 1980s and early 1990s. For example, between 1961 and 1994 the number of macrozoobenthic species on the Romanian shelf fell from 70 to 14 (Zaitsev and Mamaev, 1997) and there was mass mortality of benthic species (between 100 and 200 tonnes of organisms per km² of shelf) because of oxygen depletion. Recently, there has been some relaxation from the pressure of eutrophication on the Black Sea ecosystem. The first signs of recovery have been seen in the pelagic communities, while the zoobenthic communities are responding more slowly.

Functioning of the Black Sea ecosystem has profoundly changed since the early 1970s under cumulative effects of excessive nutrient enrichment (eutrophication), strong cooling/warming events (climate change), over-exploitation of pelagic fish stocks, and a population explosion of gelatinous species.

While very little published information is available on the effects of ocean acidification on specific marine organisms inhabiting the Mediterranean and Black Seas, many taxonomic groups present in these two semi-enclosed seas are also found in high latitude regions where many OA studies are presently focused. Thus lessons learned there will assist the design of experiments in Mediterranean and Black Seas. It is also agreed that one of the main consequences of an elevated *p*CO₂ in seawater will be a reduction in the rate of biogenic calcification in many marine species (e.g. corals, coccolithophores, pteropods, foraminifera, benthic molluscs, echinoderms and crustacea). Research on warm water corals and calcifying phytoplankton (coccolithophores) indicated controversial responses of calcification rate with increased atmospheric CO₂ concentrations and concomitant decreased CaCO₃ saturation state in seawater. Beyond these two groups of organisms, there is very little published information about potential impacts on other calcifying species of ecological (pteropods, foraminifera) and additional economic (molluscs, echinoderms and crustacea) importance. Similar studies in the Mediterranean and Black Seas are only in their infancy.

4.2. Biological response to OA (based on experiments in the laboratory or in mesocosms)

4.2.1. Pelagic organisms

Coccolithophores: coccolithophores are responsible for approximately half of the global CaCO₃ production and can cause feedbacks to atmospheric CO₂ cycling (Milliman, 1993; Ridgwell *et al.*,

2007). They have a well documented continuous fossil record since the Mesozoic and in the Mediterranean provide a unique tool for Cenozoic paleo-reconstructions. Coccolithophores can also serve as mineral ballast which plays a role in the export of particulate organic matter to the deep ocean (Klaas and Archer, 2002; Ziveri *et al.*, 2007). The effects of changes in coccolith calcification on carbon export in the Mediterranean and Black Seas are completely unknown but deserve attention: for pelagic benthic organisms, coccolithophores provide a major mechanism for food transfer from the surface to depth.

There have been controversial results from culture experiments (Riebesel *et al.*, 2000; Langer *et al.*, 2006; Iglesias-Rodriguez *et al.*, 2008) on the impact of increased CO₂ on coccolithophores calcification. Experimental studies suggest a species-specific response of coccolithophores most probably due to artificial conditions used in the laboratory and this may not be relevant for field extrapolation. As only four species have been tested so far, the question of how many types of responses coccolithophores display, and whether these types are related to phylogenetic relationships, geographical distribution or life cycle phases, remain open. While *Emiliana huxleyi* is the dominant coccolithophore species in the Mediterranean Sea, the coccolith carbonate production is dominated by several other species depending on the individual mass of the coccolithophores (Young and Ziveri, 2000). To date no study has investigated the effect of OA on a Mediterranean coccolithophore species. However the large variability observed in size, morphology and carbonate mass of some key species (Ziveri *et al.*, this volume) suggests a calcification response to the wide range of carbonate ion and CO₂ concentrations in surface waters (Schneider *et al.*, 2007).

While no detailed studies have been performed on coccolith carbonate production in the Black Sea, preliminary studies have shown very low species diversity, with *Emiliana huxleyi* again largely dominating. SeaWIFS data for 1998-2002 suggest that the Black Sea consistently experiences high reflectance patches of *Emiliana huxleyi* throughout the basin each year during the May-July period (Cokacar *et al.*, 2001; 2004). How OA can affect the major bloom activity in this basin is still unknown. However, the presence of a thick layer of coccoliths in surface sediments of the Black Sea (Hay, 1988) demonstrates that coccolithophores have also been present over the last few thousand years.

Pteropods: these planktonic molluscs are very common in the Mediterranean. Some species of pteropods produce a calcareous shell made of aragonite. Because of the higher solubility of aragonite in comparison to calcite – the constituent of foraminifera and coccolithophores calcareous structures – they are believed to be among the first planktonic organisms that will be threatened by ocean range (Orr *et al.*, 2005). Because of the difficulty to culture these organisms, no published studies are available to date on the impact of low pH on their growth and calcification rates. Ongoing research in high latitudes areas and in the Mediterranean Sea will hopefully fill this gap in coming years.

Planktonic foraminifera: to the best of our knowledge, the effect of OA on foraminifera that are present in the Mediterranean and Black Seas has not been investigated. Most of the work on foraminifera in the Mediterranean Sea has been obtained from the fossil record. Interestingly, previous studies have shown changes of foraminifera shell mass during periods of sapropel formation (Principato *et al.*, 2006) when a lower saturation state of the deep photic zone with respect to calcite has been suggested. Changes in foraminifera shell weight due to changes in surface water carbonate chemistry have also been suggested by several studies in other regions (Barker and Elderfield, 2002).

Jellyfish: there is some evidence (Attrill *et al.*, 2007), which is controversial (Haddock, 2008; Richardson and Gibbons, 2008), that OA in conjunction with increasing temperature may lead to higher abundances of jellyfish over the next century. During the last two to three decades, jellyfish blooms have increased throughout the Mediterranean Sea, but the lack of adequate time-series on pH and jellyfish blooms frequencies prevents the resolution of this question.

4.2.2. Benthic organisms

The Mediterranean Sea hosts a high biodiversity and a relatively high number of endemic species (Fowler, this volume), together with a number of unique benthic ecosystems characteristic of its subtropical and temperate basins. Among these are the vermetid reefs, *Posidonia oceanica*

meadows, coralligenous reefs, deep sea coral assemblages and *Cladocora caespitosa* bioherms, all of which are biodiversity hot-spots that might undergo dramatic changes under ocean acidification conditions.

The coralligenous reefs: coralligenous reefs are characterized by numerous calcareous algae (Corallinaceae and Peyssonneliaceae, red algae) whose thalli are linked together, building a hard and heterogeneous structure of a few centimeters to a few meters in thickness, extending over rocky substrates between the *Posidonia oceanica* meadows and the muddy bottoms of the continental shelf. This remarkable landscape harbors some 650 invertebrate species and at least 30 fish species. A number of studies have shown that calcareous algae are highly sensitive to ocean acidification conditions (Hall-Spencer *et al.*, 2008; Hall-Spencer and Rodolfo-Metalpa, this volume; Kuffner *et al.*, 2008). Coralline algae were absent from most experimental OA systems as well as from the nearshore CO₂ vent zone off Ischia. Inability of calcareous algae to form bio-constructions might result in loss of the many species dependent on it. As photosynthetic organisms, these algae may benefit from increased *p*CO₂ but their role as framework builders is crucial for the well-being of many other organisms. Failure to do so, together with natural eroding processes by physico-chemical forces and bio-eroding organisms, may shift the present balance from net buildup to net dissolution.

Posidonia meadows: *Posidonia oceanica* is a seagrass endemic to the Mediterranean Sea and it constitutes meadows which are the basis of a highly diverse and productive ecosystem. The *Posidonia* ecosystem is threatened by anthropogenic perturbations such as OA. Under increased *p*CO₂, *P. oceanica* reaches a higher density, with a lower epibiont coverage (Hall-Spencer and Rodolfo-Metalpa, this volume), which may be advantageous for a photosynthetic engineering organism. On the other hand within the low pH zone *P. oceanica* appears more susceptible to grazing due to lack of calcareous epiphytes.

Vermetid reefs: vermetids are sessile gastropods distributed in tropical and subtropical seas. The vermetids have a tubular, irregularly uncoiled shell cemented (in the adult organisms) to hard substrates. In the Mediterranean, two reef-building vermetids, *Vermetus triqueter* and *Dendropoma petraeum*, thrive in intertidal or shallow subtidal zones forming dense aggregates of colonial individuals with very high densities. Vermetid reefs have been described from rocky shores in the southern Mediterranean, from the Gibraltar Strait and southeastern Spain to the Levantine Basin. The importance of these reefs lies in the species richness they sustain, in their rarity, and in the physical protection from erosion they give the shoreline. Ocean acidification may affect both the calcifying abilities of vermetid gastropods and affect buildup to balance dissolution. Furthermore, the coralline algae involved in consolidating the vermetid's buildup are very sensitive to decreased pH as discussed above, and this may also reduce buildup rates. To date, no study is available on the effect of OA on this important bio-constructor in the Mediterranean Sea.

Deep water corals: in the Mediterranean Sea the deep-water corals community is like an oasis in the desert. In fact, the complex three-dimensional structure built by corals provide distinct ecological niches for a great number of species (Rogers, 1999), including crustacea and fish species of economic interest, such as *Aristeus antennatus* and *Helicolenus dactylopterus* (Tursi *et al.*, 2004). The white azooxanthellate coral reefs may function as nurseries for many deep-water species and as centres of spreading for the associated fauna, having positive "spill-over" effects on the deep-water demersal resources (Fossa *et al.*, 2002). This unique assemblage has developed in a very stable environment, leading to a relatively low resilience of these organisms to change. While it is expected that changes in seawater temperature or pH in deep water environments will lead to the demise of the high biodiversity through various mortality events, both the effects of OA and environmental factors on deep water corals are unknown, especially for Mediterranean species.

Cladocora banks: *Cladocora caespitosa*, perhaps the most important constructional species in the Mediterranean Sea, can form structures comparable to tropical reefs (Schuhmacher and Zibrowius, 1985; Kružić and Požar-Domac, 2003). Some large banks are found in the Ligurian (Morri *et al.*, 1994), Adriatic (Kružić and Požar-Domac, 2003) and Aegean seas (Kühlmann, 1996). Several informal reports suggest a decrease in the population size and spatial distribution of this species in the Mediterranean. Possible causes of this decrease may be global processes such as global

warming and acidification, or more local causes such as competition with invading species (algae and invertebrates).

Benthic molluscs: several studies have shown significant negative impacts of decreasing pH on mollusc species, including reduced growth and calcification rates as well as metabolic depression (Gazeau, this volume). Recent studies also revealed health and immune system deterioration following several weeks exposure to moderate hypercapnia, observations which suggest the incapacity of these ecologically and economically important species to adapt to low pH conditions. Such long-term investigations of moderate hypercapnia are obviously lacking for Mediterranean and Black Sea species.

Echinoderms: echinoderms play major ecosystem roles as keystone predators and grazers (Paine, 1966; Estes and Palmisano, 1974), in bioturbation and remineralisation (Ambrose *et al.*, 2001), and as food sources for commercial fish (e.g. *Limanda limanda*; Duineveld and Noort, 1986; Mattson, 1992) and crustaceans (e.g. *Nephrops norvegicus*; Baden *et al.*, 1990). Studies have shown a negative OA impact on growth, survival and physiology of adult sea urchins (e.g. Shirayama and Thornton, 2005; Miles *et al.*, 2007; Dashfield *et al.*, 2008); controversial results such as increased regeneration ability in brittle stars (Wood *et al.*, 2008); or species-specific response (see below). However, the impact of OA on Mediterranean species remains poorly investigated. Only Hall-Spencer and Rodolfo-Metalpa (this volume) reported a drastic decrease of echinoderms abundance in the Mediterranean under acidified conditions. More experiments are needed.

Larval stages: whereas some species spend their entire life cycle in the plankton (holoplankton), many organisms are planktonic for only a part of their life cycle, usually during the larval stage (meroplankton). Larval stages are the most sensitive to environmental stresses (Pörtner and Farrell, 2008), but surprisingly only few studies have focused on early developmental stages. In the Mediterranean region, data are available only for the sea urchin *Paracentrotus lividus* (Dupont and Thorndyke, this volume), showing an overall positive impact (higher metamorphosis success), and for cephalopods and fishes, reflecting both a morphological and physiological impact (Jeffree *et al.*, this volume). Nevertheless, data available for other regions (Dupont *et al.*, 2008; Dupont and Thorndyke, this volume; Gazeau, this volume; Havenhand *et al.*, 2008; Kurihara *et al.*, 2004) clearly demonstrate that OA impact on early developmental stages is difficult to predict, and is species-specific even in closely related taxa. For example, sea urchins show positive, neutral or negative survival responses to OA depending on the species. If OA induces calcification problems in some species (e.g. brittle stars), other calcifying larvae do not have difficulty constructing a normal skeleton (e.g. sea urchins) under near future pH conditions. Both positive (e.g. better survival in tunicates) and negative (e.g. delay in development in sea stars) impacts are also observed on non-calcifying species (Figure 3).

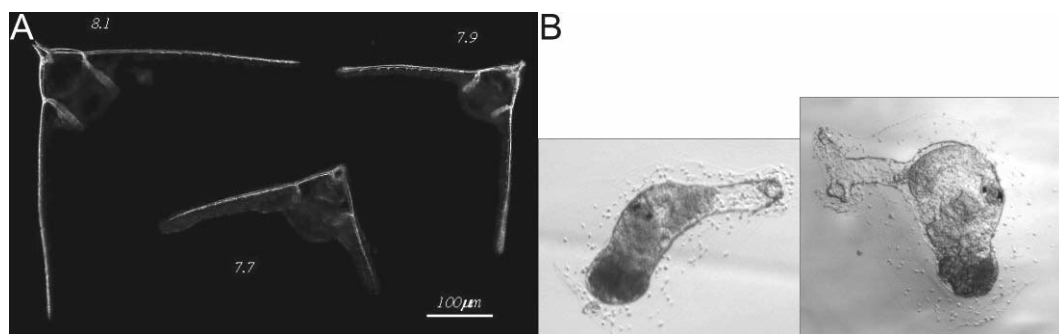


Figure 3. (A) Development abnormalities in larvae of the brittlestar *Ophiothrix fragilis* raised at different pH (polarized light is used to highlight skeletal malformations). At low pH, larvae are smaller and more asymmetric, leading to 100% mortality after 8 days (Dupont *et al.*, 2008). (B) Comparison of two-days old larvae of the tunicate *Ciona intestinalis* raised in control (pH 8.1, left) and acidic (pH 7.7, right) conditions. At low pH, larvae are larger and show a faster developmental rate.

[photos by Sam Dupont, Department of Marine Ecology, Göteborg University, Sweden]

The main conclusion from all available biological data is that OA impact is species-specific, showing apparent contradictions and paradoxes. Adequately predict the impact of future ocean

acidification on organisms and ecosystems in the Mediterranean and Black Seas will require investigating consequences on a wide range of local species and ecosystems and so better understand the reasons for such paradoxical results.

4.3. Field studies on community and ecosystem responses

The response of marine ecosystems to increased atmospheric CO₂ levels has been extrapolated mainly from results of short-term laboratory experiments in which pH or CO₂ levels were manipulated in aquaria and examined over short timescales. Although these experiments have shown species-specific responses, there is a general consensus of an overall detrimental effect of acidification on calcifying marine organisms. The long-term effects of decreasing seawater pH on ecosystems are largely unknown. However, a unique field study using natural submarine CO₂ vents has facilitated assessing OA effects on a shallow water ecosystem. Gas emitted from the sea bottom acidified seawater in the vent zone to levels that are expected to be seen by the end of this century and beyond (Hall-Spencer *et al.*, 2008; and Hall-Spencer and Rodolfo-Metalpa, this volume). Because CO₂ vents at this site have existed for centuries to millennia, benthic species have acclimatized or adapted in their natural ambient to chronic acidified conditions. Preliminary results show that long-term exposure to low pH has dramatic impacts on calcifying organisms, but that a suite of organisms in this vent community are resilient and may benefit from such conditions. This chronic acidification stress may ultimately lead to major shifts in species composition.

Consistent with responses derived from laboratory studies, typical rocky shore communities with abundant calcareous organisms, such as coralline algae, corals and sea urchins, shifted to communities lacking calcifiers. Their sensitivity to decreased pH was consistent with the mineral composition of their shells or skeletons. Therefore, calcifiers like snails, limpets and barnacles were more resilient also at very low pH (<7.7). This shift in the community composition showed no indication of adaptation or replacement of sensitive species by others capable of filling the same ecological niche, and it confirms the need for concern about the future ecology of our seas in a high-CO₂ world.

In general, it seems that photosynthetic, non calcifying species such as green and brown algae, or the sea grass *Posidonia oceanica* that forms one of the most important and rich ecosystem in the Mediterranean Sea, might directly take advantage of the acidified conditions by increasing their photosynthetic rates or, indirectly by the exclusion of other competitors for space. Some invasive alien species, which cause damage to ecosystems worldwide, may also thrive at high CO₂ levels. Although investigations on this topic are at their infancy, it seems that acidified conditions do not affect fish behaviour and abundance. Indeed, adults and juveniles of species like *Sarpa salpa*, *Labrus* sp. and *Scorpaena porcus* were found at very low pH.

This field study indicates that nearly a third of such species might disappear at the lower pH levels predicted for the future, constituting the first evidence of a potential shift in marine community structure due to ocean acidification. This unique and useful natural laboratory will be used extensively to test the response and sensitivity of a large range of benthic species to long-term acidification exposure. Another way to test impact at a larger scale is to use mesocosm experiments, “simple assemblages or even complex subsets of whole ecosystems”.

Future research priorities should include other CO₂ vent zones that are abundant in other parts of the Mediterranean Sea (e.g. the Aeolian Islands and Aegean Sea): they can be used as field laboratories to obtain data on ecosystem responses to OA.

5. SOCIO-ECONOMIC IMPACTS OF OCEAN ACIDIFICATION – REGIONAL ASPECTS

In view of the intensity of aquaculture and artisanal fisheries in the Mediterranean, to any adverse effects of OA on the viability or productivity of living resources could have high socio-economic significance. Based on current knowledge, these adverse effects would be expected first on bivalves, but could also extend to sea urchins and cephalopods. Collaboration between natural resource scientists and economists will be required to assess the scale of possible economic losses associated with seafood depletions due to OA.

Adverse impacts of OA on the geographical extent, structure and biodiversity of coral reef habitats could be expected to affect the species dependent upon them, particularly those fishes that use them as nurseries for their early life stages, with attendant economic losses to regional communities. There is growing evidence that marine benthic communities are subjected to change as a result of local perturbation, over-exploitation and global climate change, primarily global warming and ocean acidification. Mediterranean Sea ecosystems are likely to experience similar processes although we lack long-term data indicating so (Astraldi, 1995; Bianchi, 2007; Fowler, this volume). Mediterranean Sea ecosystems and benthic communities were shaped by long term geological and ecological processes but recent observations such as the occurrence of warm-water species in shallow waters of the northern Mediterranean suggests rapid ongoing changes (CIESM, 2008). This is also supported by records of elevated temperature of deep water (Bethoux *et al.*, 1990) and current warming trends (CIESM Hydrochanges Program <<http://www.ciesm.org/marine/programs/hydrochanges.htm>>). In light of these changes and the projected increase in carbon dioxide concentration, it is extremely important to understand how ocean acidification might affect Mediterranean Sea ecosystems.

There is some evidence, although controversial, that OA may lead to increases in the abundances of jellyfish over the next century. This effect would further exacerbate the current problems of their occurrences in those coastal regions that are used in the summer as prime tourist destinations. Despite current adaptive approaches at some of these locations to reduce exposure to medusae (e.g. the use of netted barriers), the repeated occurrence in the future of medusae in even higher abundances would be expected to ultimately deter tourists, with the associated losses of revenues and employment opportunities for coastal populations.

Additionally, the outbreaks of harmful algal blooms (HAB) in the Mediterranean and Black Seas that have been linked to climate change and eutrophication effects may be further exacerbated by OA. There are no HAB species that are calcifiers, and they may obtain a relative ecological advantage by OA over their calcifying algal competitors. Accordingly, enhanced harmful algal blooms would adversely affect tourism as well as regional fisheries and aquaculture production, consumption and export potential.

In addition to direct effects on particular high-value seafood species, any effect of OA on organisms necessary or important in the diet of seafood species, for example calcified pteropods, would also adversely affect their abundances.

Loss or degradation of coral reef environments due to OA could also have negative socio-economic impacts in those regions that particularly attract tourists for recreational diving, bathing and viewing from underwater observatories or glass-bottom vessels.

6. WORKSHOP RECOMMENDATIONS: GAPS AND FUTURE WORK

6.1. Main gaps

- lack of long term data (monitoring studies, in particular enhanced time-series studies) on impacts of OA on marine organisms and ecosystems;
- understanding of cumulative and combined stresses of environmental factors including OA on marine ecosystems;
- changes in the chemistry and biogeochemical cycling of carbon and carbonate;
- standardization of the experimental methods used for measuring the basic parameters, and employing AQC analyses;
- examination of issues related to OA using a multidisciplinary approach;
- in general data and research on OA in the Mediterranean Sea are very recent, while comparable data and published material for the Black Sea are presently lacking;
- socio-economic impact and public awareness (what is the perception of the problem among citizens and decision makers, and how to improve that?).

6.2. Recommendations for future research

Data availability: targeted research on OA impacts in the Mediterranean Sea is quite recent and robust data are very few. There is a need for basic data on survival, reproduction, fitness, etc.,

in a variety of Mediterranean species. In particular, more focus should be placed on ecologically and economically important and relevant species such as pelagic tunicates (salps), jellyfish, bivalves, crustacea, echinoderms and fish. Data and published information are lacking for the Black Sea, where a thorough review of relevant information should first be carried out, followed by initiation of scientific studies on OA.

Time series: long time-series studies are too few for an area like the Mediterranean Sea under high anthropogenic and environmental pressure. It is particularly important to have time-series data on the seawater carbonate chemistry: some data are available for the Western Mediterranean but they are scant or basically lacking for the Eastern Mediterranean and the Black Sea. Essential basic parameters such as T, S, O₂, DIC, and TA are recommended to be measured on a biweekly/monthly basis. Therefore, new directions and developments for monitoring the changes due to ocean acidification should be undertaken.

Biological aspects: there is an urgent need for basic biological data (survival, reproduction, fitness) on the impacts of OA on Mediterranean and Black Sea species. To give a realistic overview and allow future prediction, it is important to:

- Be not too prescriptive in species/ecosystem selection. Biological impact of OA is species-specific, and it is therefore inappropriate to extrapolate from a few model species or from one region to another. A wide range of keystone species should be investigated. For example, it is time to move beyond assessing only calcification effects and also investigate non-calcifying species such as tunicates (e.g. salps) and jellyfish that may benefit from OA.
- Assess the long term impact. To understand the real impact of OA on species, it is essential to embrace the whole life cycle and not just focus on the direct impact on one facet of the life history (e.g. adult stages). For example, multigeneration studies should be performed to assess microevolution and adaptation.
- Use realistic conditions for experiments. Relevant abiotic (e.g. pH, temperature) and biotic (e.g. food concentration, density) conditions should be taken into account in experimental designs to give a realistic view of the impact of OA in the field. The synergetic impact of these parameters with other stressors (e.g. pollutants) should also be investigated.
- Take intraspecific variability into account. Species and population adaptive potential should be investigated. More ecophysiology studies are needed. OA can impact organisms at many different physiological levels, not only calcification but also ion transport, neural and muscle activity, calcium transport and signalling, ciliate movement, etc. These functional aspects should be correlated with ecological parameters (habitat, life-history, etc.).
- It is dangerous to extrapolate from laboratory experimental results on few species to community level where there is an overall lack of evidence of OA impact. To take into account ecosystem processes such as biotic interactions, large scale experiments (mesocosm, FOCE approach, etc.) and field studies in natural environments under the influence of acidification (e.g. CO₂ vents) should be performed.
- Amongst marine calcifiers the calcification mechanisms differ considerably. For example, some organisms produce their skeleton as an epidermal secretion with direct contact to the environment (e.g. molluscs, crustacea, worms) while others have enclosed skeletons with at least one epithelium integument (e.g. echinoderms). To better understand the effects of pH and the saturation state of carbonate on calcifiers, there is a need for more information on the basic calcification mechanisms in many different taxa, including corals for which these mechanisms are poorly known.

Methodology: one must refine already established tools and develop new geochemical ones (e.g. $\delta^{11}\text{B}$, B/Ca ratios) to study biogenic carbonate in order to reconstruct the chemistry of CO₂ during the pre-industrial period at different depths in the water column. Understanding historical changes in ocean chemistry will greatly assist in predicting future oceanic conditions.

Modeling: assessing future changes in Mediterranean and Black Sea biogeochemistry in response to climate change and increasing atmospheric CO₂ levels calls for an integrated

modeling approach at the regional scale. Such models should also include the synergistic effects of multiple stressors (e.g. pH, temperature, nutrient loads, etc.).

Socio-economic perspective: the economic implications at regional and local scales should be investigated by way of collaboration between natural resource economists and scientists so as to evaluate potential economic detriment resulting from OA impacts on the fisheries and tourism industries.

ACRONYMS:

OA: Ocean Acidification

HAB: Harmful Algal Bloom

C_T : Total Inorganic Carbon; $C_T = [CO_2] + [HCO_3^-] + [CO_3^{2-}]$

A_T : Total Alkalinity

pCO_2 : Partial Pressure of CO_2

C_{org} : Organic Carbon

IPCC: Intergovernmental Panel on Climate Change

I - WORKSHOP COMMUNICATIONS

Ocean acidification issues in the Mediterranean and Black Seas: present status and future perspectives

Scott W. Fowler

ABSTRACT

The semi-enclosed Mediterranean and Black Seas have many unique oceanographic and biogeographical features which could either serve to exacerbate or reduce future effects of increased $p\text{CO}_2$ and ocean acidification (OA). Despite the fact that these seas have been the intense focus of marine research activities for decades, few studies on the effects of OA have been carried out in the region and specific OA studies with Mediterranean species and communities are only in their infancy. The existing literature relevant to this region is reviewed, specific gaps in knowledge are identified and types of OA studies needed in the future are emphasized. Owing to the overall lack of specific OA research in the Black Sea, a marine area under severe anthropogenic and environmental stress, targeted studies on future impacts of OA on its fragile marine ecosystem are urgently needed.

BACKGROUND

It is now well documented that the uptake of anthropogenic carbon dioxide (CO_2) by the ocean is resulting in acidification of its waters on a global scale (Caldeira and Wickett, 2003; 2005; Orr *et al.*, 2005). In fact at an atmospheric CO_2 concentration of ~ 380 ppmv today, the average pH of surface waters has dropped by 0.1 unit since the pre-industrial period, and simulations for the next century are predicting a further reduction from 0.3-0.5 pH units depending upon which Intergovernmental Panel on Climate Change (IPCC) CO_2 emission scenario is used for the calculation (Caldeira and Wickett, 2005). Nevertheless concerns about acidification as a threat to the marine environment are fairly recent and, consequently, there is little known about the present and future impacts of increased ocean acidification (OA) on marine organisms and, more importantly, on community structure and entire ecosystems. However, it is generally agreed that one of the main consequences of an elevated partial pressure of CO_2 will be a reduction in the rate of biogenic calcification in many marine species (e.g. corals, coccolithophores, pteropods, foraminifera, benthic molluscs, echinoderms and crustacea) brought on by a lower sea water pH and a concomitant decrease in calcium carbonate (aragonite and calcite) saturation. Most research to date on this topic has focused on warm water corals and certain calcifying phytoplankton (e.g. coccolithophores), and there is now considerable evidence that the calcification rate in these species decreases with increased atmospheric CO_2 concentrations and concomitant decreased CaCO_3 saturation state in sea water (see Fabry *et al.*, 2008; Guinotte and Fabry, 2008 for reviews). Besides these two groups of organisms, there is very little information available about potential impacts on other calcifying species of ecological (e.g. pteropods, foraminifera, coralline algae) and economic (e.g. molluscs, crustacea and echinoderms) importance.

Model studies using the IPCC IS92a “business as usual” CO₂ emission scenario indicate that some areas of the subarctic North Pacific and most of the high latitude Southern Ocean will be undersaturated with respect to aragonite by the end of this century (Orr *et al.*, 2005). Based on these findings and model predictions, priority for ocean acidification (OA) studies is presently focused on high latitude and polar ocean areas since they will likely experience the greatest changes in carbonate chemistry during the coming decades. Given this current direction in OA research, similar studies and assessments of mid-latitude seas such as the Mediterranean Sea and Black Sea have received far less attention and are only now beginning to be made. Nevertheless these two semi-enclosed seas are of fundamental economic, social and cultural importance to the 26 countries and large populations surrounding them, and furthermore they display certain unique oceanographic and biogeographic features that could result in either an exacerbation or a reduction of the potential effects of OA in their waters. Thus, in the following sections, I briefly review what is presently known about acidification issues and research in the Mediterranean and Black Seas, and present some suggestions for targeted OA research which might lead to a better understanding of how these two seas will respond to increasing levels of CO₂ in the future.

THE MEDITERRANEAN SEA

The Mediterranean Sea is surrounded by 21 countries with a combined population in the catchment basin of some 235 million inhabitants sharing a coastline approximately 46,000 km long. Several oceanographic, geographic and ecological features of the Mediterranean Sea render it potentially vulnerable to anthropogenic stressors. The sea is a relatively shallow, semi-enclosed water body with only limited water exchange taking place through the Straits of Gibraltar and the entrance to the Black Sea. The Mediterranean is characterized by low precipitation, high evaporation, high salinity and alkalinity, high oxygen concentration, low tidal action and relatively low nutrient concentrations outside the inner coastal zones. Low salinity Atlantic surface water enters at Gibraltar, slowly circulates counter clockwise around the Mediterranean, gradually becoming warmer and more saline due to increased insolation and evaporation, and then exits the Mediterranean at depth through the Gibraltar Straits. One of the most unique features is its relatively high minimum temperature (~13°C) which is basically isothermal from beneath the mixed layer down to the sea bottom. The very small amplitude tides combined with a regime of weak coastal currents limit the dispersion of land-based anthropogenic substances entering coastal waters. The sea is generally oligotrophic with low nutrient concentrations that typically decrease from the western to the eastern basin. This gradient in nutrients leads to significantly lower primary productivity in the eastern basin and, together with strong summer stratification of the water column and a tight microbial loop control, results in exceptionally low exports of particulate organic carbon to the sea floor.

Despite an overall low biological productivity, the Mediterranean Sea hosts a high species diversity compared to the Atlantic. This is primarily because it is a warm sea in temperate latitudes and therefore hosts both temperate and subtropical species. The sea contains approximately 8,560 macroscopic species (between ~ 4% and 18% of all world marine species) of which more than 25% are endemic (Bianchi and Morri, 2000). There is a notable east to west trend in increasing richness of species which closely reflects climate and trophic gradients. In general, Mediterranean species are characterized by smaller individual sizes than their Atlantic counterparts. Over the last few decades a northward migration of certain species associated with warming waters has been demonstrated in several regions of the western Mediterranean (see CIESM Monograph 35, 2008). The increase in the number of tropical Atlantic species invading the northern Mediterranean may result from a combination of anthropogenic and climate factors. Increased water temperature anomalies and coastal pollution have been deemed to be causes of a number of mass mortality events, coral pathogen outbreaks, viral infections in certain species, harmful algal blooms, noxious jellyfish blooms and excessive mucilage production. A number of Mediterranean species are also endangered by human activities such as uncontrolled fishing, coastal construction causing loss of natural habitats, and severe coastal pollution in certain areas. As coastal commercial fisheries and aquaculture are highly important to many of the countries surrounding the sea, any additional environmental and anthropogenic impacts on these activities could have serious consequences not only for the marine ecosystems involved but for the socio-economic well-being of the region.

In terms of predicting future effects of climate change in the Mediterranean, based on available information some general projections have been made recently by Anadon *et al.* (2007). Evidence has accrued which documents large-scale warming in the basin and changing biodiversity in response to it. Given the depth (mean = 1,450 m), rapid deep water turnover time (40-50 yrs), and numerous endemic species of this semi-enclosed basin, it is projected that such impacts may lead to earlier changes in Mediterranean biodiversity than will occur in other European seas. However the numerous microclimates found throughout this sea make any prediction over large spatial scales difficult and, consequently, to date most effects of climate change or climate anomalies have been identified only on regional scales.

THE BLACK SEA

The Black Sea, the world's largest land-locked and anoxic sea, is located in a semi-arid climatic zone and is surrounded by only six countries; however, its entire catchment area is over five times the size of the sea and includes areas of 21 countries with a combined population approaching some 200 million inhabitants. The northwestern portion of the sea receives the discharge of the largest rivers (e.g. Danube, Dnepr and Dniestr) flowing into it. At the surface sea temperatures vary from 0°C in the northwestern coastal area in winter to 25°C in the western sector during summer, and at 1,000 m depth remain a near constant 9°C throughout the year. The Black Sea's only connection to other major seas is with the Mediterranean via the Bosphorus, the Sea of Marmara and the Dardanelles. The latter link is shallow and narrow, and the inflow of highly saline Mediterranean water is outweighed by the outflow of fresher Black Sea water on the surface. This water deficit is compensated by the freshwater discharge of rivers, and these two inflows of water have resulted in a marked stratification of the water column. The brackish surface water, with an average salinity of ~17.5-19 per thousand and a calcite saturation state of 7.5 (Goyet *et al.*, 1991; Hiscock and Millero, 2006), overlies denser water (mean salinity ~22 per thousand) at depth. The resultant permanent halocline situated between 100 to 200 m is a distinguishing feature of the Black Sea. Furthermore, the density difference between the two water masses coupled with a lack of well-defined vertical currents prevents vertical mixing and any penetration of oxygen from surface to the bottom. Hence, over the centuries the sinking of surface-derived particulate organic matter (POM) and its subsequent decomposition at depth has left the Black Sea permanently anoxic below depths of 150-200 m, resulting in 90% of its total water volume being anoxic. Under these anoxic conditions further POM degradation uses oxygen bound in nitrates and sulfates, with the latter chemical reduction resulting in the formation of hydrogen sulfide. Due to the production of H₂S which has contaminated 90% of the total water body, only anaerobic bacteria can survive there.

The most biologically productive areas in the Black Sea include the northwestern and northeastern portions of the basin which receive enhanced nutrient loads from inflowing rivers and undergo sufficient vertical mixing. In general, the brackish nature of the sea's waters limits the numbers of species present. With respect to fisheries, of the 180 described fish species in the Black Sea, more than half are also present in the Mediterranean. During the last three decades overfishing coupled with abrupt climatic changes, intense eutrophication and the invasion of foreign species have led to profound alterations in the food chain and at times near total collapse of the standing stocks of all trophic levels above the phytoplankton. Prominent among these periodic catastrophic events was the dramatic increase in medusa (*Aurelia aurita*) during the 1970s and 1980s, and the explosion of the ctenophore *Mnemiopsis leidyi*, both of which caused a major reduction in second trophic level grazers such as fish larvae and copepods (Kideys, 2002). Given the sensitive nature of its trophic structure, its unique marine chemistry, and the tremendous impacts that overfishing and eutrophication have had, it is clear that the Black Sea is under considerable anthropogenic and environmental stress.

At present there appear to be no firm projections about how climate change will affect the Black Sea in the future. The information at hand indicates the occurrence of well-defined oscillations during the last century, and it is expected that these oscillations and perhaps certain catastrophic events will continue in the future superimposed on a general warming trend. However making accurate predictions of climate change effects is complicated by the fact that, compared to other European seas, the Black Sea is influenced by several interconnected oceanic and atmospheric

patterns including the North Atlantic Oscillation. Furthermore this region has been affected by external anthropogenic factors such as overfishing, eutrophication and pollution. Hence, the present condition of the Black Sea suggests that any additional stressors, e.g. acidification from rising atmospheric CO₂ concentrations which might negatively impact its waters, could have profound effects on its entire ecosystem.

REGIONAL OCEAN ACIDIFICATION STATUS

It should be emphasized at the outset that research regarding biological and ecological effects of OA is in its infancy and the extent of current knowledge is limited. This is particularly true for the Mediterranean and Black Seas since these semi-enclosed water bodies are linked with major mid-latitude oceanic areas that are not projected to experience the early effects of acidification as will occur in high latitude seas. Nevertheless many groups of organisms present in the Mediterranean and Black Seas are also found in high latitude and polar regions where many OA studies are presently focused. Thus, any information on biological effects gleaned from high latitude studies could have important implications for certain Mediterranean and Black Sea ecosystems, and vice versa.

There is a body of evidence mainly from laboratory culture experiments that increasing acidification will reduce calcification in marine organisms (see Fabry, 2008; Fabry *et al.*, 2008; Guinotte and Fabry, 2008 for reviews). This includes groups such as corals, foraminifera and coccolithophores. The latter calcareous phytoplankton group, which accounts for approximately one third of the total marine CaCO₃ production, includes the globally important species *Emiliana huxleyi*, the most common coccolithophore in the Mediterranean Sea and particularly in the Black Sea where large blooms of this species often occur (Tyrrell *et al.*, 2008). Earlier controlled laboratory experiments as well as semi-enclosed mesocosm experiments using *E. huxleyi* and other coccolithophore species have demonstrated reduced rates of calcification in response to elevated pCO₂ in sea water (Riebesell *et al.*, 1993; Zondervan *et al.*, 2001; Sciandra *et al.*, 2003; Engel *et al.*, 2005; DeLille *et al.*, 2005), although in one experiment with *Coccolithus pelagicus* no change in calcification with elevated pCO₂ was noted, whereas in another using *Calcidiscus leptoporus* under similar conditions, an initial increase in calcification was observed followed by a decrease (Langer *et al.*, 2006). More recently it has been reported that, when carefully simulating the dissolved inorganic carbon concentration and resultant pH in experiments by bubbling CO₂ through the culture media, the calcification rate and net primary production in *E. huxleyi* are significantly increased by high pCO₂ (Iglesias-Rodriguez *et al.*, 2008). Based on the highest pCO₂ values used (750 ppmv) which followed one scenario for the end of the century (Caldiera and Wickett, 2003), their results suggest that the projected pCO₂ levels and calcite saturation state would be unlikely to affect the metabolic balance, organic carbon production and calcite precipitation in this coccolithophore species. Their experimental findings were corroborated by evidence from high latitude sediment cores which indicates that, since pre-industrial times, there has been an approximate 40% increase in coccolith calcite mass in the subpolar North Atlantic.

From the above-cited studies, it is apparent that different coccolithophore species can exhibit different calcification responses under varying pCO₂ levels. Furthermore, to explain the divergent results with *E. huxleyi*, it has been suggested that this cosmopolitan species is composed of several different varieties with distinct morphological, physiological and genetic properties. Therefore, in terms of obtaining response data specific to this region, it would be important to test such responses in *E. huxleyi* as well as other prevalent coccolithophore species (e.g. *Gephyrocapsa ericsonii*, *Gephyrocapsa oceanica*, *Gephyrocapsa protohuxleyi*, *Syracosphaera pulchra*, and *Florisphaera profunda*; Knappertsbusch, 1993) collected from the Mediterranean and Black Seas. Investigations of deep sea cores using methodologies similar to those described by Iglesias-Rodriguez *et al.* (2008) would also be instrumental in establishing both coccolithophore species composition and any changes in coccolith mass and productivity that have taken place in these seas during the Anthropocene period.

Although enhanced pCO₂ levels and decreased pH may not reduce calcification in some coccolithophores, it could lead to variations in elemental stoichiometry in their cells which is known to influence “food selection” by second trophic level grazers. Depending upon food chain

patterns, such changes in phytoplankton preference by grazing zooplankton could in turn affect the export flux of carbon (POC and carbonate) to the deep sea (Anderson and Pondaven, 2003; Engel *et al.*, 2005). Given the fact that depending upon the season, coccolithophores represent the majority of the phytoplankton standing stock in the Mediterranean, any shift in chemical composition of the coccolithophores, or in phytoplankton composition in general, may have a major impact on the quality and quantity of the sinking particulate carbon (POC and CaCO_3) exported to depth.

While “tipping points” in response to calcite undersaturation may vary among calcifying phytoplankton like coccolithophores, other important phytoplankton species may respond metabolically to acidification in different ways leading to shifts in competitive advantages which could have significant ecological and biogeochemical consequences (Fabry, 2008). For example outbreaks of harmful algal blooms (HABs) in the Mediterranean and Black Seas have been closely linked to climate change and eutrophication effects, but as yet there is no evidence indicating a cause-effect relationship between OA and HABs. Although no HAB species are calcifiers, it is possible that OA could alter phytoplankton metabolism and community composition in a manner resulting in a favourable shift in HAB dynamics. Any subtle effects of acidification on phytoplankton, as well as on other organisms, may be masked by local environmental changes brought about by multiple anthropogenic stressors; therefore it is important to unravel the separate effects of all these stressors before the actual impact of OA on phytoplankton and particularly HABs can be elucidated.

Another case in point is determining the underlying causes for the increase in medusa blooms that have occurred throughout the Mediterranean and Black Seas over the last several years. Attrill *et al.* (2007), studying continuous plankton recorder data from western North Sea waters between 1971 and 1995, found a negative relationship between jellyfish abundance and pH, and suggested that in response to increasing OA the frequency of jellyfish will increase over the next century. However, that conclusion has recently been placed in doubt based on a much broader survey from the same general area and a large portion of the North Atlantic which failed to find any significant relationships between jellyfish abundance and surface layer pH in those regions (Richardson and Gibbons, 2008). Despite the fact that no cause and effect relationship has been established between jellyfish blooms and a reduction in sea water pH, it is still an open question whether a lowering of pH might trigger a physiological mechanism leading to enhanced production of medusae or other noxious marine species. Therefore if similar types of long-term monitoring measurements exist in areas of the Mediterranean and Black Seas where major jellyfish outbreaks have occurred over the last few decades, a search for correlative relationships might help establish whether or not reduced pH is actually benefiting certain marine organisms.

Most studies investigating the effects of OA calcification processes have dealt with warm water corals, and experimental results have clearly shown that increased $p\text{CO}_2$ can significantly reduce coral calcification. Nevertheless, from the geological record it is not clear whether such a reduction in calcification would lead to the eventual demise of the coral colonies. This aspect has been recently tested experimentally with two scleractinian Mediterranean species, *Oculina patagonica* and *Madracis pharencis*, that were continually exposed for 12 months to a low (7.3-7.6) and ambient (8.0-8.3) pH regime (Fine and Tchernov, 2007). Control colonies maintained their health and integrity throughout the experiment whereas those held under low pH conditions underwent complete skeleton dissolution and polyp elongation and increase in size. Remarkably, all the decalcified polyps survived during the year and, when transferred back to ambient pH conditions, they calcified and reformed colonies. These experimental findings suggest that Mediterranean corals and perhaps other species could survive the pH regime that is predicted for the next century due to increased $p\text{CO}_2$. Testing this hypothesis using coral colonies in the field would be extremely difficult, but examining it using a wide range of coral types (e.g. warm and cold water species) under properly simulated conditions and over extended time periods could provide better insights into how corals can adapt to large scale environmental change of sea chemistry. *Oculina patagonica* exists over a range of differing environmental conditions (e.g. temperature, light, food types, anthropogenic stressors) in both basins of the Mediterranean, and thus would be an excellent model species to examine calcification and physiological response to low pH under different environmental conditions.

It should be kept in mind that the Mediterranean is a warm temperate sea in which the minimum water column temperature does not normally go below $\sim 13^{\circ}\text{C}$. Furthermore average sea water temperatures can be expected to rise in view of recent results from model studies which predict that increasing greenhouse gas concentrations will dramatically increase the occurrence of hot extremes by 200 to 500% throughout the Mediterranean region (Diffenbaugh *et al.*, 2007). Since increasing sea water temperatures along with concomitant shifts in the distribution of certain species have been well documented in the Mediterranean, such temperature effects also need to be taken into consideration when considering the effects of increasing $p\text{CO}_2$ on calcifying and non-calcifying organisms. Such abiotic factors can control the structure and function of marine ecosystems and little is known about their synergistic or antagonistic interactions as regards metabolic responses and effects on the calcification mechanism. However, there is now good evidence with a scleractinian coral, *Stylophora pistillata*, that temperature and $p\text{CO}_2$ significantly interact synergistically to control calcification, and that the rate of calcification in this species could decrease by 50% by the end of this century given the predicted increases in sea water temperature and $p\text{CO}_2$ that will occur during this period (Reynaud *et al.*, 2003). Clearly more effort in studying the combined effects of temperature and $p\text{CO}_2$ on Mediterranean species (e.g. calcifying plankton, scleractinian and soft corals, fish, commercial molluscs, etc.) is needed for improving our predictions about the future effects of OA on the various marine ecosystems in the Mediterranean Sea.

While there is a growing body of laboratory experimental results demonstrating the negative effects of OA on marine calcifying organisms, there is an overall lack of solid field evidence to support the conclusions and projections drawn from these experimental data. It is even more difficult to find evidence that such observed effects in target organisms might in fact alter entire ecosystems and biodiversity. However, Hall-Spencer *et al.* (2008), studying the benthic ecosystem of coastal volcanic CO_2 venting sites off the coast of Ischia in the Tyrrhenian Sea, have made an important advance in our understanding of marine ecosystem response to chronic high CO_2 and low pH exposures. In the area of the vents they measured a gradient of pH decreasing from ambient (8.1-8.2) just outside the vent zone to as low as 7.5 (mean 7.8-7.9) near the vents. Outside the vent zone a typical rocky shore community with abundant calcareous organisms was present, but near the CO_2 vents there was a marked shift to a community lacking scleractinian corals and impoverished in coralline algae and sea urchins. High $p\text{CO}_2$ acidification effects on the local gastropod community were evident as demonstrated by individuals with partially dissolved shells and a complete lack of juvenile gastropods at the vent sites. In these areas where calcareous organisms were impacted, there was a notable increase in sea grass production and non-calcareous algal cover, indicating that photosynthetic organisms such as sea grass and certain brown algae can take advantage of this high CO_2 niche. While coastal CO_2 vents are not exact analogues of CO_2 inputs on a global scale, this study provides direct evidence and essential information on high $p\text{CO}_2$ effects on organisms and ecosystem structure over spatial and temporal scales that are otherwise difficult to obtain experimentally. Shallow water CO_2 venting sites are common along the active volcanic arcs in the Tyrrhenian and Aegean Seas (Dando *et al.*, 1999), and owing to their relatively easy accessibility, much more use should be made of these natural laboratories to assess the long term effects of elevated $p\text{CO}_2$ and acidification on benthic community structure.

In addition to these shallow-water, CO_2 -vent communities comprised of corals and other calcifying species, deep-sea cold-water coral reef ecosystems are abundant along the northern rim of the Mediterranean extending from the Adriatic and Aegean Seas to the northwestern basin (Roberts *et al.*, 2006). There is an overall lack of studies concerning OA effects on these deeper coral ecosystems, but it has been suggested that a lower carbonate saturation state in deeper waters could render these cold-water corals more vulnerable to OA impact than tropical species. Furthermore, certain modelling studies have projected that by the end of this century, the shoaling of the aragonite saturation horizon (i.e. the depth at which aragonite dissolves) will put at risk some 70% of the known locations of deep-sea bioherm-forming scleractinian corals (Guinotte *et al.*, 2006). In fact a recent projection of reductions in surface water aragonite saturation state indicates values of < 3 in the western Mediterranean basin and 3 - 3.5 in the eastern basin by 2060-2069 when $p\text{CO}_2$ reaches 517 ppmv (Guinotte and Fabry, 2008). This scenario poses a serious threat to Mediterranean corals if one considers that in the sea at present, carbonate accretion approaches zero

or is negative at aragonite saturation states of 3.3 (Hoegh-Guldberg *et al.*, 2007). Thus, studies comparing responses of Mediterranean deep water scleractinian reefs to increased $p\text{CO}_2$ with those of coral colonies living in shallow coastal waters, including reefs exposed directly to naturally high $p\text{CO}_2$ levels from volcanic venting, should offer useful insights into the relative vulnerability of these two Mediterranean coral ecosystems to future increases in $p\text{CO}_2$ levels.

Corals are not the only calcifiers under the threat of OA; there are many Mediterranean nearshore mollusc species, some of high economic value, which could be impacted by global rise in CO_2 as well as by localized inputs from activities on land. Mussels are highly valued bivalves which are being cultured by many countries around the Mediterranean basin. As a shallow water benthic species they often encounter abrupt short-term changes in O_2 , $p\text{CO}_2$ and pH levels. Recent studies by Michaelidis *et al.* (2005) on the long-term effects of moderate hypercapnia on growth and acid-base balance in the Mediterranean mussel *Mytilus galloprovincialis* have demonstrated that a reduction of pH to 7.3 caused acidosis in mussel tissues, but the degree to which this occurs was limited by a concomitant dissolution of the shell. Furthermore over a three-month period shell growth in the hypercapnic mussels was reduced by 55%, most likely due to a 65% reduction in metabolic activity compared to the control group. While the authors point out that these dramatic reductions in growth and metabolism were observed at a pH value which is not expected to occur for ~ 300 years, they suggest that such effects would materialize much before that value is reached. Similarly designed laboratory experiments carried out with the Mediterranean seabream, *Sparus auratus*, led to a reduction in blood plasma and intracellular pH as well as other changes in various enzymatic activities in muscle tissue (Michaelidis *et al.*, 2007). Nevertheless the resultant acidosis was compensated by the fish increasing its cellular and plasma bicarbonate levels. This latter metabolic response, also noted in other Mediterranean fish (Cecchini *et al.*, 2001), may be one reason why among marine organisms fish are believed to be the most tolerant to effects of OA (see Fabry *et al.*, 2008 for review). Such laboratory experiments using targeted species confirm the premise that a lowering of pH to levels predicted by various increased $p\text{CO}_2$ scenarios can have severe negative impacts on shelled molluscs and significantly alter acid-base balance and enzymatic functions in commercially important fish from Mediterranean waters.

In parallel with research on the physiological responses to hypercapnia in individual species, an equally important avenue of research is experimentally examining Mediterranean community response to realistic $p\text{CO}_2$ levels that are projected to occur by the end of the century. In a month-long semi-controlled mesocosm experiment, Bourret *et al.* (2007) have studied the influence of a doubling of $p\text{CO}_2$ (350 vs 700 ppmv) on a natural microbial food web (bacteria, diatoms and protozoa) inhabiting a coastal Mediterranean lagoon. These treatments were tested against an abiotic 700 ppmv CO_2 control, and two separate models considering assimilation of total inorganic carbon and assimilation of only dissolved CO_2 by phytoplankton were employed. The results showed that doubling of $p\text{CO}_2$ was buffered by photosynthesis in that the sea water pH actually increased due to CO_2 uptake by the phytoplankton. Modelling of the food web indicated that only a weak increase in phytoplankton biomass occurred in response to elevated $p\text{CO}_2$, but there were increases in carbon fluxes, notably via phytoplankton excretion and sinking dead organisms. Although difficult to interpret, such mesocosm experiments are often the only means by which the influence of different variables on specific endpoints in complex natural communities can be assessed. Thus, they serve as a useful bridge between controlled laboratory experiments on the effects of $p\text{CO}_2$ on targeted marine species and longer term field observations in natural ecosystems such as those carried out in the Mediterranean CO_2 vent fields.

GENERAL CONCLUSIONS

This brief review of regional OA research failed to identify any research papers dealing specifically with acidification issues in the Black Sea and its ecosystem. At first glance this may seem somewhat surprising for such a fragile sea area; however, it may simply reflect present day research priorities arising from the mix of other anthropogenic stressors known to affect the Black Sea (see Kideys, 2002). Nevertheless, the unique anoxic state of 90% of its waters and the relatively thin layer of water (0-200 m maximum) in which marine organisms are subject to other environmental and anthropogenic stresses suggest that additional impacts from OA could trigger “tipping points” in the ecosystem at a much earlier stage than in the Mediterranean Sea. In this respect targeted

research on how OA stressors may affect the fragile ecosystems in the Black Sea is urgently needed. In any case the existing information base on OA suggests that the warmer, mid-latitude Mediterranean and Black Seas are not oceanic areas where the effects of OA will first be felt. Furthermore, the Mediterranean is strongly supersaturated with respect to calcite and aragonite at all depths, and it has a high total alkalinity, typically $\sim 2,600 \mu\text{mol kg}^{-1}$ and homogenous throughout the water column, which would act to buffer the acidification due to increasing $p\text{CO}_2$ levels (Schneider *et al.*, 2007). Likewise, the calcite saturation state in surface waters of the Black Sea is very high (~ 7.5) and appears to be relatively constant throughout the year (Tyrrell *et al.*, 2008). These alkalinity characteristics alone would tend to slow the onset of a global increase in OA. Nevertheless, other regional features such as a large and growing population encompassing both these sea areas, rapid urbanization and industrialization, and the very long coastal zone receiving inputs of acidic compounds from the rivers and atmosphere suggest there is potential for increased land-based sources of anthropogenic acidification (e.g. Doney *et al.*, 2007) that would not be present in the more pristine high latitude and polar zones. Therefore, continued research on the effects of OA in the Mediterranean Sea and, in particular, the Black Sea should be encouraged.

EPOCA (European Project on Ocean Acidification) and other research efforts devoted to the Mediterranean Sea

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Since the beginning of the industrial revolution, about one third of the CO₂ released in the atmosphere by anthropogenic activities has been absorbed by the world's oceans, which therefore play a key role in moderating climate change. As CO₂ reacts with sea water, it generates dramatic changes in the carbonate chemistry, including decreases in pH and in the concentration of carbonate ions. Average surface water pH values on the total scale are in an accelerating decline: 8.3 during the last glacial maximum, 8.2 just prior to the industrial era, and 8.1 at present; surface pH may reach 7.8 by the end of this century. This pH level will probably be unprecedented in several tens of millions of years and the changes in carbonate chemistry are happening at a speed 100 times greater than has ever been seen before. The impacts of this phenomenon, known as ocean acidification, on marine ecosystems are only poorly known. As oceans continue to acidify, there is an increasing risk of loss of biodiversity and of profound ecological shifts. One of the most likely consequences is the slower growth of organisms forming calcareous skeletons or shells, such as corals and mollusks.

The EU FP7 Integrating Project EPOCA (European Project on Ocean Acidification; <<http://epoca-project.eu>>) was launched in May 2008 with the overall goal to advance our understanding of the biological, ecological, biogeochemical, and societal implications of ocean acidification. The EPOCA consortium brings together more than 100 researchers from 27 institutes and nine European countries. The budget of this four year long project is 15.9 M€, including 6.5 M€ from the European Commission.

The research interests of EPOCA are divided into four themes:

- Theme 1 focuses on past and present spatiotemporal changes in ocean chemistry and biogeography of key marine organisms. Paleo-reconstruction methods are used on several archives, including foraminifera and deep-sea corals, to determine past variability in ocean chemistry (carbonate, nutrients, and trace metals) and to tie this variability to present-day chemical and biological observations.
- Theme 2 aims to quantify impacts of ocean acidification on marine organisms and ecosystems. Molecular, physiological and ecological approaches are used to study climate-relevant biogeochemical processes, including calcification, primary production and nitrogen fixation. Laboratory and field perturbation experiments focus on key organisms in terms of their ecological, biogeochemical, or socioeconomic importance. The potential for adaptation

and acclimation is also assessed. EPOCA focuses on areas where ocean acidification is thought to strike first (Arctic Ocean and the North Atlantic).

- Results from themes 1 and 2 are integrated in biogeochemical, sediment, and coupled ocean-climate models to project future responses of the Earth system to ocean acidification. Special attention is paid to feedbacks of physiological changes on the carbon, nitrogen, iron, and sulfur cycles and how these changes affect and will be affected by future climate change.
- Theme 4 synthesizes results obtained in themes 1, 2 and 3 to evaluate uncertainties, risks and thresholds (“tipping points”) related to ocean acidification at molecular, cellular and organismal levels and from local to global scales. It also assesses the decrease in CO₂ emissions required to avoid these thresholds and describe the change to the marine environment and Earth system, should these emissions be exceeded.

Interactions between the different themes is both ways (Figure 1). For example, theme 3 exploits information from theme 2 to help predict future changes in ocean biogeochemistry and ecosystems, but results from theme 3 also feed back into theme 2 by providing critical information on the expected temporal and spatial changes of ocean acidification and thus enable meaningful experimental designs.

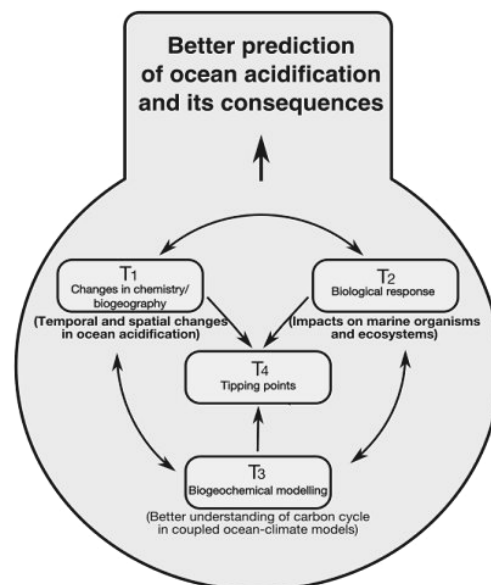


Figure 1. Schematic picture of the four research themes of EPOCA and how they interact.

Strict budget constraints forced the project to focus most EPOCA activities in the North Atlantic and the Arctic. Some areas of the Arctic Ocean will become undersaturated with respect to aragonite as early as 2018. Two major experiments will be carried out in Svalbard in 2009 and 2010 to investigate the biological response of benthic and pelagic organisms.

EPOCA's research efforts cannot cover to a great extent several important areas, including the Mediterranean Sea and the Black Sea. EPOCA Mediterranean research actions are limited to a few observational and biological response actions. Within theme 1, carbonate chemistry is continually measured at the time-series station “Dyfamed” off the French coast and at “Point B” in the bay of Villefranche sur mer (France). Theme 2 activities in the Mediterranean include the CO₂ vents studies by Jason Hall-Spencer and colleagues near Ischia Island in Italy (see Hall-Spencer and Rodolfo-Metalpa, this volume) and experiments on the impact of ocean acidification on Mediterranean deep-sea corals, microbial interactions and pteropods.

There is a general lack of knowledge of the extent of ocean acidification and its consequences in the Mediterranean and Black Seas. It is therefore critical to:

- Assess the present carbonate chemistry and its projection in the future. To this effect, time-series stations should be established in the Eastern Mediterranean and the Black Sea.
- Investigate past episodes of ocean acidification.
- Resolve the current discrepancies related to the response of zooxanthellate scleractinian corals. In contrast to tropical species, the only Mediterranean species investigated so far does not seem to be affected at environmentally-relevant pH levels. However, there is a strong negative impact of elevated temperature. This also demonstrates the absolute need to investigate synergistic and antagonistic effects.
- Investigate early life stages.
- Go beyond calcification and investigate processes such as primary production, nitrogen fixation and other key processes of the nitrogen cycle, microbial processes, and biogeochemical cycling.
- Determine the socioeconomic impacts and cost of ocean acidification for Mediterranean countries.

Temporal variations of anthropogenic CO₂ concentrations in the Mediterranean Sea

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ABSTRACT

The unique data set of monthly observations collected over more than a decade at the time-series station DYFAMED (between Nice and Calvi), allows us to estimate the temporal variation of the distribution of anthropogenic CO₂ in the western Mediterranean Sea. The results indicate 1) that the concentrations of anthropogenic CO₂ are much higher than those found in the Atlantic Ocean, and 2) that the temporal trend for anthropogenic CO₂ is decreasing and following that of dissolved oxygen. The decrease of anthropogenic CO₂ resulted from an invasion of old water masses. This, indicates that small changes in water circulation due to anthropogenic pressures (global warming, river dams, etc.), may provoke significant impacts on anthropogenic CO₂ penetration in the Mediterranean Sea, which in turn will affect its acidification.

BACKGROUND

The most recent report (<<http://www.globalcarbonproject.org/carbontrends/index.htm>>) from the GCP-Global Carbon Budget team indicates that the Annual mean growth rate of atmospheric CO₂ was 2.2 ppm per year in 2007 (up from 1.8 ppm in 2006), and above the 2.0 ppm average for the period 2000-2007. The average annual mean growth rate for the previous 30 years was about 1.5 ppm per year. This increase brought the atmospheric CO₂ concentration up to 383 ppm in 2007, that is 37% above the concentration at the start of the industrial revolution (about 280 ppm in 1750). The present concentration is the highest during the last 650,000 years and probably during the last 20 million years.

Emissions increased from 6.2 PgC per year in 1990 to 8.5 PgC in 2007, a 38% increase from the Kyoto reference year 1990. The growth rate of emissions was 3.5% per year for the period of 2000-2007, an almost four fold increase from 0.9% per year in 1990-1999.

Natural CO₂ sinks absorb 55% of all anthropogenic carbon emissions slowing down climate change significantly. Natural land and ocean CO₂ sinks have removed ~4.8 PgC per year of all CO₂ emitted from human activities during the period 2000-2007. The size of the natural sinks has grown in proportion to increasing atmospheric CO₂. However, the efficiency of these sinks in removing CO₂ has decreased by 5% over the last 50 years, and will continue to do so in the future. That is, 50 years ago, for every ton of CO₂ emitted to the atmosphere, natural sinks removed 600 kg. Currently, the sinks are removing only 550 kg for every ton of CO₂ emitted, and this amount is falling.

The global oceanic CO₂ sink removed 25% of all CO₂ emissions for the period 2000-2007, equivalent to an average of 2.3 PgC per year. The size of the CO₂ sink in 2007 was similar to that in the previous year but lower by 0.1 PgC compared to its expected increase from atmospheric CO₂ growth. This was due to the presence of a La Nina event in the equatorial Pacific. The Southern Ocean CO₂ sink was higher in 2007 compared to 2006, consistent with the relatively weak winds and the low Southern Annular Mode. An analysis of the long term trend of the ocean CO₂ sink shows a slower growth than expected over the last 20 years. The decline is attributed to the strengthening of the winds (due to global warming and the ozone hole) around Antarctica which enhances the ventilation of natural carbon-rich deep waters.

Anthropogenic CO₂ emissions have been growing about four times faster since 2000 than during the previous decade, this despite efforts to curb emissions in a number of countries which are signatories of the Kyoto Protocol. Emissions from the combustion of fossil fuel and land use change almost reached the mark of 10 billion tones of carbon in 2007. Natural CO₂ sinks are growing, but more slowly than atmospheric CO₂ which has been growing at 2 ppm per year since 2000. This is 33% faster than during the previous 20 years. All of these changes characterize a carbon cycle that is generating stronger climate forcing and sooner than expected. The efficiency of natural sinks has decreased by 5% over the last 50 years (and will continue to do so in the future), implying that the longer it takes to begin reducing emissions significantly, the larger the cuts needed to stabilize atmospheric CO₂.

This fast growing anthropogenic forcing at the global scale is also visible at the regional scale. For instance, over the second half of the 20th century, the urban population of the Mediterranean coastal regions increased by a factor superior at 2.5. Consequently, the Mediterranean Sea is affected by all kinds of anthropogenic pressures such as changing riverine inputs, increasing fisheries, varying air-sea gas exchanges, etc.

Important changes in the circulation and physical properties of the Mediterranean Sea have already been observed (decrease of the sea level – Tsimplis and Baker, 2000; appearance of a new deep water formation site in the southern Aegean Sea – Roether *et al.*, 1998; increases of salinity and temperature in intermediate and deep waters of the western Mediterranean – Bethoux *et al.*, 1990). Similarly, its chemical composition is also changing with increase of phosphates and nitrates in the deep layers of the western basin; Bethoux *et al.*, 2002.

Despite a significant research effort since the '90s to study the penetration of anthropogenic CO₂ (C_{ant}) in the global ocean, relatively little is known about the cycle of inorganic carbon in coastal areas and in particular in regional seas like the Mediterranean Sea.

Since the residence time for the Mediterranean waters is very short (16 and 50 years for the Algero-Provencal basin and the eastern basin, respectively) compared to 250 years for the deep waters of the North Atlantic, the anthropogenic CO₂ is expected to have invaded the whole Mediterranean Sea.

What is the present distribution of anthropogenic CO₂ concentrations in the Mediterranean Sea? Will the seawater C_{ant} concentrations increase with time at the same rate as that in the atmosphere? Or will the Mediterranean Sea behave like the Southern Ocean, decreasing its atmospheric CO₂ uptake rate with time?

In the Mediterranean Sea one time-series station at the DYFAMED (DYnamique des Flux Atmosphériques en MEDiterranée <<http://www.obs-vlfr.fr/sodyf/home.htm>>) site located in the central part of the Ligurian Sea (43°25 N, 7°52 E), between Nice and Calvi did operated for 15 years from 1993 to 2007. However the properties of the CO₂/carbonate system were only measured there during the years 1998, 1999, 2003, 2004, 2005, 2006, 2007.

We used this unique temporal data set to estimate the distribution and the temporal evolution of C_{ant} in the northwestern Mediterranean Sea. Due to the lack of CT and AT data during certain periods, we first reconstructed the temporal evolution of the CT and AT profiles using interpolation procedures similar to that developed by Goyet and Davis (1997). Then, using the interpolated data set, we estimated the distribution of C_{ant} over more than a decade (1993-2005) at the DYFAMED site using the simple TrOCA approach (Touratier *et al.*, 2007).

In practice, since C_{ant} cannot be directly measured in seawater, it is necessary to estimate it using a model. At present several models based on various hypotheses exist (Brewer, 1978; Gruber *et al.*, 1996; Goyet *et al.*, 1999; Touratier and Goyet, 2004a,b; Waugh *et al.*, 2006; Touratier *et al.*, 2007) and it is difficult to assess which one is the most appropriate (Coatanoan *et al.*, 2001; LoMonaco *et al.*, 2005; Touratier *et al.*, 2005).

Here the choice of the TrOCA approach was guided by its simplicity and by the data set available. Most of the other approaches currently used to estimate C_{ant} in the ocean require the knowledge of additional properties such as the CFCs that are not available at this DYFAMED site, and could not be applied here.

Thus, C_{ant} is estimated with the following equation (Touratier *et al.*, 2007):

$$C_{\text{ant}} = \frac{O_2 + 1.279 \left[C_T - \frac{1}{2} A_T \right] - e^{\left(7.511 - (1.087 \times 10^{-2}) \theta - \frac{7.81 \times 10^5}{A_T^2} \right)} }{1.279} \quad (1)$$

where θ is the potential temperature ($^{\circ}\text{C}$).

RESULTS AND DISCUSSION

Compared with the Atlantic Ocean, the Mediterranean Sea is warm ($T > 12^{\circ}\text{C}$ throughout the water column) and salty ($S \sim 38$). The concentrations of C_T and A_T are also high (ranging from 2,400 $\mu\text{mol.kg}^{-1}$ to 2,450 $\mu\text{mol.kg}^{-1}$ and 26,500 $\mu\text{mol.kg}^{-1}$ to 2,550 $\mu\text{mol.kg}^{-1}$; about 5% higher than in the Atlantic Ocean).

From 1993 to 2006, the temperature and salinity data indicate a significant increase with time at depths greater than 1,000 m. For instance at 2,000 m depth the rates of increase for θ and S reach values of $5 \cdot 10^{-3} \text{ }^{\circ}\text{C} \cdot \text{year}^{-1}$ and $2 \cdot 10^{-3} \text{ year}^{-1}$, respectively. However due to a large influence of the seasonal variability in the surface layer, there are no clear trends for depths $< 1,000$ m.

Yet, the concentrations of both C_T and A_T increased by approximately 5 $\mu\text{mol.kg}^{-1}$ at a depth of 500 m, while those for O_2 and C_{ant} decreased by 20 $\mu\text{mol.kg}^{-1}$ and 15 $\mu\text{mol.kg}^{-1}$, respectively.

The decreasing trend of C_{ant} at the DYFAMED site was unexpected, especially because during this same period of time the concentration of C_T increased (C_{ant} represents a small fraction of C_T).

The decreasing concentration of C_{ant} below 500 m is strongly and positively correlated with the O_2 content. Recently, Joos *et al.* (2003) pointed out that significant reductions of O_2 (from a few $\mu\text{mol.kg}^{-1}$ up to 30 $\mu\text{mol.kg}^{-1}$) were detected in all major oceans. According to these authors, the anthropogenic radiative forcing is the main cause of the observed decreases in dissolved O_2 . This is also confirmed by both the observation-based analyses and the global ocean models.

In order to explain a decrease of O_2 of $\sim 5 \mu\text{mol.kg}^{-1}$ in the Eastern Mediterranean Deep Waters (EMDW) during a four-year period (1995-1999), Klein *et al.* (2003) suggested that it was a consequence of the Eastern Mediterranean Transient event (EMT) which took place in the late '80s, when approximately 20% of the EMDW was replaced by dense surface waters originating from the Aegean Sea.

An increasing state of North Atlantic Oscillation (NAO) would also tend to reduce the precipitations (Tsimplis and Josey, 2001) and change wind stress (Samuel *et al.*, 1999), thus affecting the whole Mediterranean Sea circulation especially during the recent decades.

In addition, the damming of important rivers like the Nile and the Ebro river (in the early '60s), directly or indirectly led to a significant decrease of the freshwater load (reduction by more than 90% and 60% for the Nile and the Ebro rivers, respectively). Most of these changes provoked an increase of the Mediterranean Sea surface salinity and had significant impacts on water circulation especially at the sites of Deep Water Formation.

Many uncertainties still subsist. However, it is clear that the distribution of physical and chemical properties (S , θ , C_T , A_T , O_2 , and C_{ant}) at the DYFAMED site have been influenced by processes that occurred more than 10 years ago, in the Eastern Mediterranean. The observed decrease of O_2 and C_{ant} is due to an invasion of older waters in the intermediate and deep layers at this site.

Even if the concentration of C_{ant} observed at the DYFAMED site around the 400 m - 800 m depth range has slightly decreased with time over the last 13 years, the level remains surprisingly high, especially compared with the Atlantic waters near Gibraltar. Since acidification in the oceans mainly results from the accumulation of C_{ant} in the Mediterranean Sea, there is a significant reduction in pH from the surface to the bottom. Thus the Mediterranean Sea which plays an important role in the uptake of anthropogenic CO_2 , is a key area to observe the effects of anthropogenic impacts (increases of temperature, CO_2 , pH, etc.) on marine chemistry and ecosystems.

Past and present variations in the C-cycle and related changes in eastern Mediterranean C deposition and preservation

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CO₂ can be produced by either enhanced atmospheric CO₂ content and subsequent fluxes into the ocean such as the present and future situation, or by enhanced hydrocarbon / organic matter fluxes and subsequent deep-water degradation such as during past large-scale anoxic events. This CO₂ will dissolve in water thus making it more acidic and resulting in ocean acidification. For studying the effects of such enhanced CO₂ content of the ocean, the initial cause is not relevant.

To the best of my knowledge no high-precision published data exist on present-day Mediterranean acidification. Studying the paleo records is an alternative approach to evaluate potential effects and consequences of ocean acidification in the Mediterranean ocean laboratory. There is no essential difference between the burning of fossil fuels and the degradation of organic matter or hydrocarbons, all leading to CO₂ as the final product. Degradation of organic matter during its descent to the seafloor is a continuous process that only for areas with high surface water productivity leads to an extreme and distinctly noticeable deviation in water column characteristics. The common observation is an oxygen minimum zone (OMZ) such as in the NW Indian Ocean with oxygen depletion for the greater part of the water column from less than 200 to more than 1,000 m, and concomitant deviations in nutrients and in the carbon cycle. In deep ocean water it is the relative magnitude of oxidant (e.g. oxygen) supply by ventilation and reductant (organic matter) supply by primary productivity that determines the state of the ocean water with respect to redox potential, C system, and pH. A stagnant deep water mass and an enhanced organic matter supply will thus lead to the accumulation of enhanced levels of deep water CO₂ content. To assess the potential scale and severity of the present anthropogenic CO₂ generation we need to see if in the past, events of similar magnitude have ever occurred.

Is there a paleo event with which we can compare the present and near-future development of CO₂-related ocean acidification? One of the most comparable events is in fact the PETM event of ~ 55 Ma years ago, that is thought to be related to a similar quantity and rate of release of CO₂ as may be liberated in the near future (compare numbers given by Dickens *et al.*, 1995 and others; ...2,000 to 4,000 Gt C). The exact origin for the rapid CO₂ increase during the PETM is as yet not fully ascertained. Mainly on the basis of the extreme δ¹³C excursion at this event, degradation of large amounts of hydrocarbons such as methane (possibly from gas hydrates; Dickens *et al.*, 1995) and potentially organic matter has been postulated to have initiated this extreme CO₂ excursion. Whatever the origin, the massive amount of CO₂ seems undisputed as well as its dramatic effects on the deep ocean carbonate dissolution (e.g. Zachos *et al.*, 2005). From the event onward, the latter continued to be important in the deep ocean for periods up to 100,000 years. This fits quite

nically with modeling efforts that have indicated that PETM and potentially future C-system CO₂ recovery may take a period of ~ 100,000 years. Not only changes in ocean chemistry have occurred during the PETM but also a pronounced reduction in marine biodiversity. Many bottom-dwelling species that form carbonate shells became extinct, possibly as a direct result of reduced pH due to ocean acidification. Similar potential ecological effects from ocean acidification can be expected due to the current increases in atmospheric CO₂. As mentioned before, these effects are likely to last for a long time.

What consequences may this have for the near future? With similar quantities and rate of supply, we may expect a similar impact on ocean chemistry. In view of the present atmospheric CO₂ source and the delayed transfer to the deep water, the largest effects will be in the surface waters. At the moment these are largely oversaturated relative to carbonate whereas the deep water in most of the oceans is undersaturated or close to saturation. A small effect in the latter will thus have a direct effect on the carbonate preservation potential. In other words, carbonate will start to dissolve in areas where until recently it was accumulating. Such dissolution will continue with increasing CO₂ content, and will also diffuse downward into the sediment. In view of the residence time for most of the oceans, it will take ~ 1,000 years before in fact all ocean deep waters will be replaced by this anthropogenic CO₂-rich water. An exception to this is the Mediterranean with a residence time of only a few decades. This area, therefore, has the potential to become a key target, ocean laboratory, for ocean acidification assessment studies.

At present the Mediterranean is one of the largest deep water basins that is saturated not only with respect to calcite but also to aragonite, aragonite being a more soluble form of CaCO₃ than calcite. Therefore, at the sediment water interface, aragonite fossils such as pteropods can be found at least down to a water depth of 3.5 km, whereas in the North Pacific the latter boundary is rather around 0.5 km. It seems therefore that for the full Mediterranean water column (perhaps with the exception of a few extremely deep holes such as Rhodes Basin: > 4 km, and Matapan Trench: > 5 km, for which I am not aware if data exist) calcite and aragonite remain saturated. The Mediterranean is a small scale ocean basin but with relatively short residence time of the deep water (50-100yr). At present, it is the sustained winter cooling of relatively shallow pre-conditioned water in the Adriatic and Aegean that leads to deep-water formation. As a consequence, any atmospheric signal such as CFC's or CO₂ is rapidly transferred to the Mediterranean deep sea.

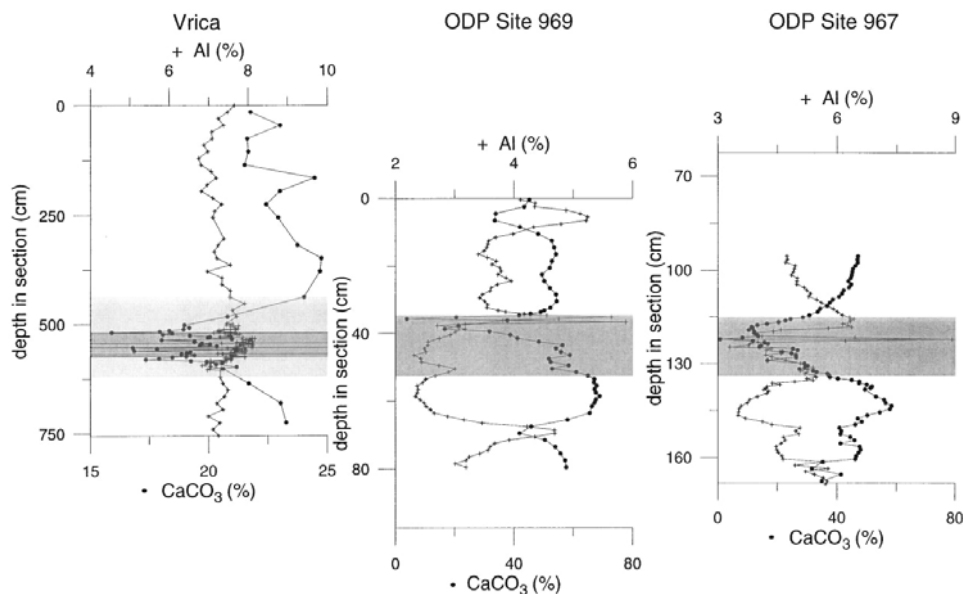


Figure 1. Corg (dots) and Ba (crosshairs) contents in Vrica, ODP Sites 969 and 967. Grey denotes the area enriched in Corg. The graphs have been arranged so that the bases of the Corg-enriched layers are aligned. The vertical axes have been scaled according to sedimentation rate: the three axes have equal length, representing 36 kyr. Note that the scale of the horizontal axes is different (after Nijenhuis *et al.*, 2001).

The eastern Mediterranean sedimentary records are known for their distinct occurrence of dark organic-rich intervals, sapropel. The latter appear in association with relatively humid climate periods, whereas the intervening periods are related to relatively arid climate conditions. The formation of sapropels has been attributed to enhanced primary productivity in the surface waters and to reduced ventilation in the deep water. Either of these would have resulted in enhanced fluxes of organic matter to the seafloor, thus to enhanced accumulating levels of CO₂ in the deep water. Clearly, such effects are observed for several intervals of enhanced productivity, and stagnation. In some of the Pliocene sapropels this has even resulted in the total removal of carbonate, whereas for the most recent sapropel, S1, the carbonate seems only partly affected (e.g. Figure 1).

This is not only observed in a reduction of the total CaCO₃ content, but also in the appearance of microfossils. Carbonate microfossils during S1 formation do not suffer from carbonate overgrowth as for those occurring after S1 formation (Crudeli *et al.*, 2006). This clearly indicates that there is a difference in the C-system between these periods. Did it ever go below saturation during this last sapropel period, i.e. from 9.8 – 5.7 kyr¹⁴C ? No systematic studies of the selective dissolution of aragonitic species have been published, but from observations we do know that in most sapropel S1 sediments, certain large aragonitic pteropods are present. However, no information is available on the pteropods exact distribution in time and with waterdepth during that period. Climate changes associated with increased atmospheric CO₂ content have been suggested, including a more humid climate in the potential deep-water source areas. If true, then this would lead to more stagnant Mediterranean deep-water, as in sapropel times, and a more frequent ventilation of intermediate, more shallow water due to reduced densities of the water forming in the source areas.

Observations of nitrogen chemistry and fluxes under high CO₂ conditions: implications for the Mediterranean Sea

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ABSTRACT

Following a review of published reports and small-scale experiments performed on coastal waters and sediments of the English Channel, the currently known impacts of ocean acidification on the microbial nitrogen cycle are presented to enable discussion of direct and indirect effects which are relevant to the biogeochemistry of the Mediterranean Sea. There is great potential for changes in the balance of the nitrogen nutrients NO₃⁻ and NH₄⁺ and between nitrogen and other nutrients including phosphorus, which ultimately infer changes in community composition and ecological status. Data from the English Channel support theoretical predictions that the balance of the NH₄⁺:NH₃ equilibrium in seawater favours NH₄⁺ under acidic conditions, which when coupled with stable PO₄³⁻ concentrations may act to increase the ratio of dissolved N:P. In surface waters, nitrifying bacteria were found to be sensitive to conditions of elevated CO₂, so that nitrification rates were reduced by approximately 30% as a result of a reduction in pH of 0.18. In coastal sediments, the removal of NO₃⁻ from overlying water, increased under high CO₂, as did the activity of denitrifying and/or annamox bacteria which coupled with changes in nitrification may lead to depletion of pelagic NO₃⁻ in absolute terms and relative to NH₄⁺. Yet robust information on many of these processes under the influence of enhanced pCO₂ is scarce. Recent research related to the fixation of carbon into organic and inorganic material, which is relevant to this study, has intensified, but has produced information that is at times contradictory. A condition which fuels our call for focussed experimental and model studies of decreasing pH in the Mediterranean basin. We tentatively propose that ocean acidification will act to increase the oligotrophic nature of the Mediterranean Sea and increase the degree of phosphorus limitation currently found, which will almost by definition, contribute to reduced productivity and carbon export.

INTRODUCTION

Elevated oceanic pCO₂ and the subsequent decrease in pH will have direct and indirect impacts on microbial nutrient cycling and carbon fixation which may fundamentally alter current biogeochemical cycles in the Mediterranean Sea. Current opinion and biogeochemical models assume that phytoplankton and prokaryotic growth is limited by nutrient availability (e.g. nitrogen, phosphorus, iron), and that carbon fixation and export are tightly coupled to these nutrient cycles through elemental stoichiometries. Under predicted ocean acidification scenarios, microbial activity and hence the biogeochemical cycles they drive may come under pressure from direct and

indirect means. Whilst research into the effects of increased $p\text{CO}_2$ on the fixation of organic and inorganic carbon has been active for several years (see Fowler; Ziveri *et al.* and references both in this volume; Orr *et al.*, 2005; Riebesell *et al.*, 2007), data that describe the impacts of ocean acidification on other key microbially driven ecosystem processes, such as nitrogen cycling, remain sparse (Blackford and Gilbert, 2007). However, by interpreting the limited empirical information in light of current understanding of marine biogeochemistry, we demonstrate the potential for future increases in the $p\text{CO}_2$ of seawater to impact both directly and indirectly on a number of marine biogeochemical cycles.

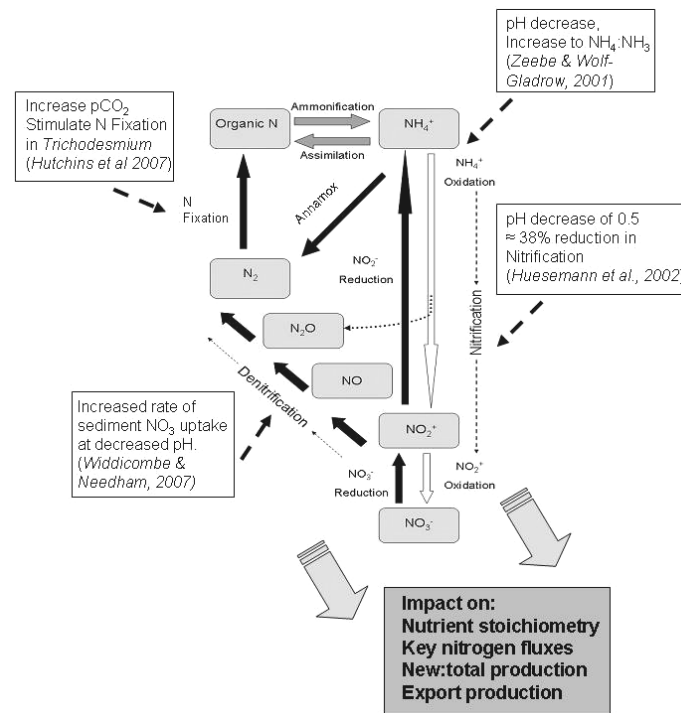


Figure 1. Currently known impacts of ocean acidification on the marine nitrogen cycle.

Direct effects:

1) The speciation of nitrogen and phosphorus nutrients shows theoretically defined shifts with changes in pH (Zeebe and Wolf-Gladrow, 2001), so that changes in nitrogen and phosphorus availability and stoichiometry may ultimately exert pressure on biological productivity and ecosystem structure.

2) Nitrification, the microbially mediated oxidation of ammonium to nitrite and nitrate, was found in a single study to be sensitive to changes in pH (Huesemann *et al.*, 2002). If this is true for other waters, then changes to nutrient availability may have diverse consequences for ecosystem structure and function, which may cause shifts between new and regenerated production in surface waters and change nitrification:denitrification coupling in sediments.

3) Recent laboratory studies have shown that N- fixation rates increased significantly with elevated CO_2 in cultured *Trichodesmium* (e.g. Hutchins *et al.*, 2007), which when applied to P deplete regions such as the Mediterranean may induce further phosphorus stress to microbial communities.

4) The flux of nutrients between benthic sediments and overlying water has been shown to be sensitive to reductions in pH (Widdicombe and Needham, 2007), which if there is unequal impact on different nutrient species will alter the balance between N and P and between NO_3^- and NH_4^- .

Indirect effects:

1) Carbon fixation has been observed to both increase and decrease following exposure to elevated concentrations of CO_2 ; this may be related to community composition and/or other environmental conditions.

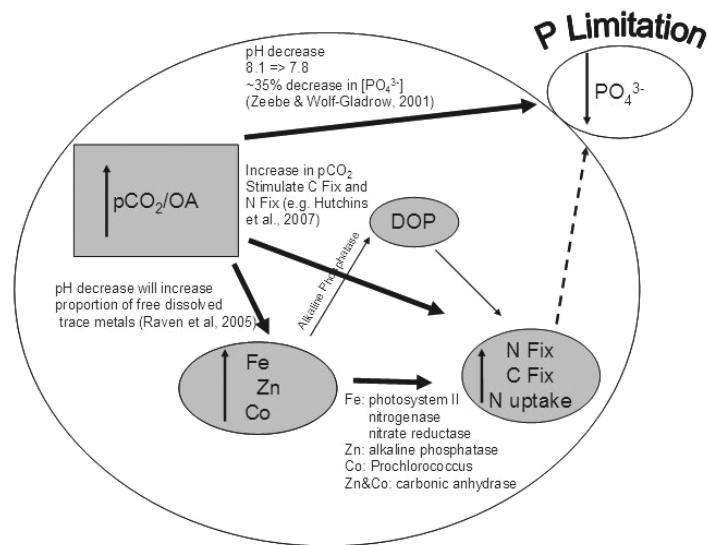


Figure 2. Potential impact of ocean acidification on trace metal and phosphorus availability which may compound changes to nutrient balance within Mediterranean waters.

2) Changes in metal speciation may fundamentally alter the availability of metalloenzymes used in numerous biogeochemical processes. e.g. nitrogenase, nitrate reductase and alkaline phosphatase, which control microbial activity and the exchange of nutrients.

3) Bioturbation (sediment mixing and bioirrigation) of marine sediments by infaunal organisms plays a significant role in the transport of nutrients across the sediment-water interface. Ocean acidification has the potential to change both the type of organisms present and the intensity of their bioturbatory activities.

The Mediterranean Sea contains some of the most nutrient-impoverished waters in the world. Although the oligotrophic to ultraoligotrophic offshore waters contrast with the eutrophic conditions experienced in some coastal areas, there is potential for ocean acidification to impact on nutrient fluxes in all areas. Due to the relatively warm conditions, dissolution of CO_2 may be tempered compared to other ocean areas and this effect may reduce the degree of ocean acidification effects below those forecast. The oligotrophic condition is associated largely with the semi-enclosed nature of the Mediterranean basin coupled with limited run-off from land, and nutrient-deplete source waters (e.g. Krom *et al.*, 1991). The productivity of marine systems is intimately connected to the supply of nutrients, and so it is of no surprise that productivity is generally low and that surface waters are characterised by being relatively clear. There is a general gradient of increasing oligotrophy in a west to easterly direction as a result of the thermohaline circulation which creates steep physicochemical gradients, with increasing salinity, temperature, and stratification towards the eastern basin. Recent forecasts for the Mediterranean Sea as a result of climatic changes in addition to ocean acidification, include drought, decline of water quality, floods, changes in soil erosion and desertification (increase dust inputs), storms, coastal erosion, changes in seawater temperature and salinity, sea level rise and biodiversity reduction. Increased stratification of the surface layer as a result of a number of these changes may reduce the amount of nutrients supplied by deep waters, which will contribute to an increase in the severity of oligotrophic conditions and an enhancement of the West-East gradients in both productivity and organic matter flux to the deep-sea.

Due to the short residence time of water in the Mediterranean Sea, it is anticipated that future changes will take place relatively rapidly (Fowler, this volume), and for a system whose productivity is tightly coupled to the supply and relative balance of inorganic nutrients this may lead to changes in ecosystem function and community composition.

The nutrient stoichiometry of the Mediterranean reveals a deficiency in P relative to N (e.g. Krom *et al.*, 2005). This relative disequilibrium is encountered throughout the year for the whole of the

Mediterranean Sea and is generally reinforced by continental influence. At the basin scale, macronutrient concentrations depend on the exchanges through the Strait of Gibraltar and through the Bosphorus, atmospheric deposition, and river discharges. In order to explain the high Mediterranean N:P ratio, several hypotheses have been proposed. These include elevated ratios in riverine inputs ($\text{NO}_3^-:\text{PO}_4^{3-}$ of 65 to 80 for the Rhone and other rivers) and Saharan dust inputs which could account for 60-100% of bioavailable -N and 30-50% of bioavailable -P in the Eastern Mediterranean with a N:P ratio of 117:1 (Kouvarakis *et al.*, 2001; Krom *et al.*, 2004). Further hypotheses which surround the disequilibria in N:P include internal hydrodynamic features such as phosphate removal by adsorption onto iron oxide rich Saharan dust particles, and the potential importance of peculiar biological processes, such as high rates of N_2 fixation.

As an area susceptible to further changes in the balance between N:P, it is quite possible that changes in nutrient stoichiometry driven by environmental perturbations could be expected to affect phytoplankton activity and diversity and, therefore, the whole ecosystem.

CURRENT KNOWLEDGE

Nutrient concentrations

The theoretical speciation of nitrogen and phosphorus nutrients shows defined shifts with changes in pH (Zeebe and Wolf-Gladrow, 2001), so that nitrogen and phosphorus availability and stoichiometry are liable to exert pressure on biological productivity and the ecosystem structure and function. Decreases in ocean pH over the predicted range are forecast to decrease PO_4^{3-} by up to 80%, and to increase the concentration of NH_4^+ and its abundance relative to NH_3 . In a Norwegian mesocosm study which followed “semi-natural” systems at three levels of pCO_2 (1x, 2x and 3x present levels), Tanaka *et al.* (2007) noted that phosphate availability was lower in the 3x system, but not statistically different from the other treatments. During a survey performed at a coastal station in the western English Channel (Figure 3), little or no change was observed following experimentally enforced pH change for both PO_4^{3-} and NO_3^- concentrations. However, over the same period and under the same experimental conditions, the predicted changes in the NH_4^+ concentration due to a H^+ ion forced shift in the $\text{NH}_4^+ \rightleftharpoons \text{NH}_3$ equilibria were observed. This resulted in modest increases in N:P ratio of 16.2 – 17.2 with the likelihood of increasing phosphorus stress in local communities. Whilst theoretical chemistry predicts decreasing PO_4^{3-} with the lowering of pH in seawater, the limited number of experimental observations provide evidence of only small, if any change. Modest increases of NH_4^+ have been recorded though and changes to the N:P ratio may act to exacerbate the P limitation of the Mediterranean basin.

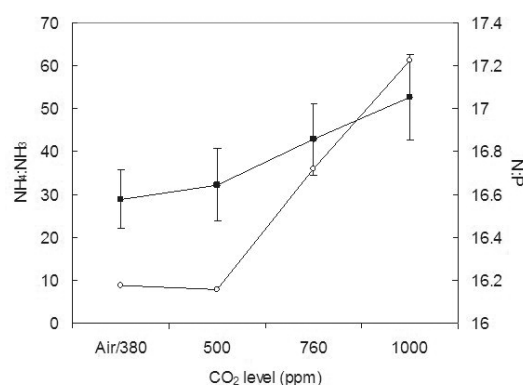


Figure 3. (■) - Ratio of dissolved NH_4^+ (determined according to Woodward and Rees, 2001) to NH_3 (theoretical value estimated from Bell *et al.*, 2007) and (○) - the resultant increase in N:P ratio over a four week period in March and April immediately prior to the spring bloom in 2008. Surface seawater samples were collected from station L4 in the western English Channel, and CO_2 levels were altered by sparging with air and CO_2 at the levels indicated for 40 minutes prior to nutrient analysis.

Nitrogen fixation

N-fixing organisms are now fully recognised to play a significant role in ocean ecosystems, as diazotrophy is acknowledged to be more widespread than was previously thought, and has been shown to be a process active in Mediterranean waters (Rees *et al.*, 2006; Sandroni *et al.*, 2007) that

may contribute significantly to new production. The Mediterranean Sea has great potential for nitrogen fixation, being an ultra-oligotrophic basin which does not exhibit Redfield stoichiometry. N:P ratios of ~28:1 occur in deep water (Krom *et al.*, 1991), and surface waters are characterised by elevated temperatures and very low inorganic nitrogen. Gruber and Sarmiento (1997) found high N^* values in the Mediterranean indicative of nitrogen fixation which they suggested could support up to 30% of the export flux. This is supported by indirect estimates from stable-isotopic evidence (e.g. Bethoux and Copin-Montegut, 1986) which indicate that up to 90% of new nitrogen could be supplied by this route.

Recent laboratory studies have shown that N- and C-fixation rates increased significantly with elevated CO_2 in cultured *Trichodesmium*. Particularly relevant to the P impoverished Mediterranean is the study of Hutchins Hutchins *et al.* (2007) found that increased CO_2 enhanced nitrogen fixation and growth rates, even under severely phosphorus-limited steady-state growth conditions. With that said, our knowledge of the distribution and activity of diazotrophs under natural conditions in the Mediterranean is limited, increased ocean acidification will further challenge our understanding, and the dangers of extrapolating from a single species to the level of community effects should be stressed.

Nitrification

Nitrification is the microbially mediated oxidation of ammonium to nitrite and nitrate and is a key process in the global nitrogen cycle. The subsequent oxidation of NH_4^+ and NO_2^- provides the pathway between the most reduced and most oxidised forms of nitrogen and may release nitrous oxide (an effective radiatively active gas which is responsible for approximately 19% of the greenhouse effect and also contributes to stratospheric ozone depletion) as a by-product. Huesemann *et al.* (2002) found that pelagic nitrifiers were sensitive to changes in pH, so that a decrease in pH of 0.5 units may decrease nitrification rate by approximately 38%. Recent work in the western English Channel (Figure 4) suggests that this effect may be as much as 75% over a pH decrease of approximately 0.3. Nitrifying bacteria obtain all their carbon requirements via the fixation of carbon dioxide, thus nitrification links the carbon and nitrogen cycles, at several levels. Nitrification in the euphotic zone is now recognised to be a key process, and may contribute a substantial level of the phytoplankton NO_3^- requirement, but as a regenerated rather than new nitrogen source. In the NW Mediterranean nitrification has been shown to provide up to 66% of phytoplankton nitrogen requirement (Bianchi *et al.*, 1999), indicating that it is an important, sometimes the exclusive, source of euphotic zone nitrate. Whilst nitrification does not affect the total nitrogen budget, i.e. there is no net loss or gain of nitrogen, it is essential in maintaining the balance between inorganic nitrogen species. Changes in the ratios of ammonium:nitrite:nitrate will alter microbial species activity and diversity, and consequently ecosystem function and the release of nitrous oxide.

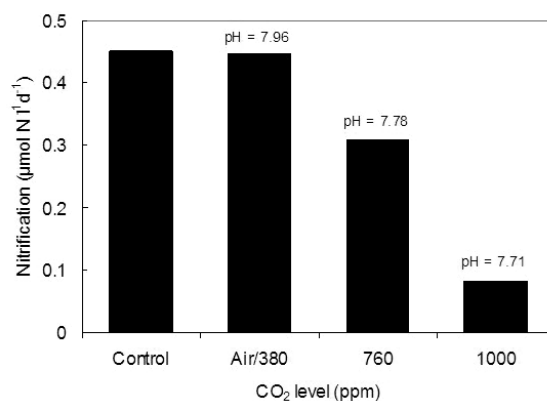


Figure 4. Nitrification rates determined by ^{14}C uptake and ATU inhibition (methods described in Rees *et al.*, 2006) at Station L4 in the western English Channel during July 2008. Seawater samples collected from 10 m were sparged with air and CO_2 at the levels indicated for 40 minutes prior to incubation to attain the pH levels shown. Incubations were initialised within four hours of sample collection and terminated after 24 hours following incubation in the dark at 15°C.

Sediment nitrogen fluxes

The exchange of nutrients between benthic sediments and overlying water has been shown to be sensitive to reductions in pH (Widdicombe and Needham, 2007), which may act to alter the balance between N and P and between NO_3^- and NH_4^+ in pelagic waters. Widdicombe and Needham found that decreasing the pH of seawater overlying sediments resulted in increased uptake of NO_3^- by sediments, an increase in the release of NH_4^+ and a decrease in PO_4^{3-} uptake. This may be explained in part by Figures 3 and 4, which suggest that the inhibition of nitrification will result in increased NH_4^+ accumulation, and increased rates of NO_3^- removal from overlying waters by denitrification. This does not necessarily indicate increased denitrification, but possibly a disabling of the nitrification-denitrification couple so that NO_3^- supply to denitrifiers must come directly from overlying and pore waters. Rates of sediment nutrient fluxes can be significantly affected by the presence and activity of bioturbating organisms and Widdicombe and Spicer (2008) have suggested that the survival and activity of such organisms could be impacted by elevated levels of CO_2 . Therefore, the potential impact of ocean acidification on sediment nutrient fluxes could be twofold: via changes in the microbes responsible for nutrient transformations and from changes in the bioturbating macrofauna responsible from the transport of solutes and oxygen across the sediment water interface.

Sediment nutrient dynamic studies in the NW Mediterranean have generally suggested a net flux of nitrogen, phosphorus and silicon into the sediments (Lucea *et al.*, 2005). Although smaller scale regional studies have revealed temporal and spatial variability in the direction and magnitude of nutrient fluxes (e.g. Denis and Grenz, 2003). For example relatively low net fluxes of ammonium and nitrate out of the sediments were demonstrated during winter months on the continental shelf in the Gulf of Lions, with a general increase in nutrient release towards the coast (Denis and Grenz, 2003). Model results confirm observations and indicate that the continental margin acts as a sink for nitrate most of the time, but during winter, with reduced phytoplankton growth and cascading of dense waters along the shelf, nitrate is released from the sediments and exported towards the open sea (Tusseu-Vuillemin *et al.*, 1998). Thus it would seem that decreases in the pH of bottom water are likely to increase the uptake of nitrogen by sediments for most of the year and modify the amount of N being released during the winter, potentially pushing the Mediterranean into N deficiency and further oligotrophy.

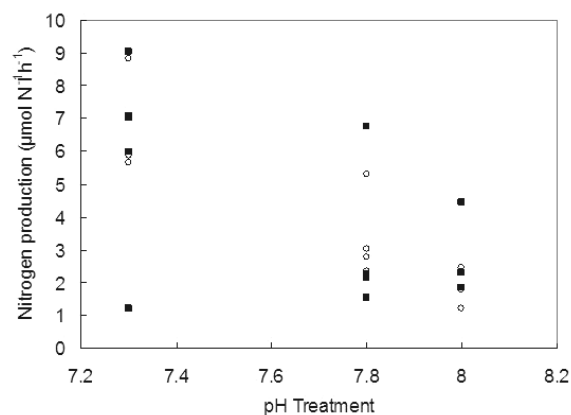


Figure 5. Nitrogen production by denitrification/anammox in Plymouth Sound sediments (○— mud, ■— muddy sand) following five week incubation under CO_2 induced changes of pH in the PML mesocosm facility (June 2007). N production was determined from ^{15}N - NO_3^- additions and the isotope pairing technique (Nielsen, 1992), which was not performed to resolve anammox from denitrification.

Carbon fixation

Although marine photosynthetic organisms are almost entirely single-celled phytoplankton that comprise less than 1 % of the total global plant biomass, they account for ~ 40 % of total global C-fixation which has been estimated to be on the order of 45 Gt C yr⁻¹. C-fixation has been studied throughout the Mediterranean by shipboard experiments (e.g. Conan *et al.*, 1999) and through the

exploitation of remotely sensed observations (Groom *et al.*, 2005). Whilst the fixation of carbon into organic and inorganic material through photosynthesis and calcification respectively has been shown to be sensitive to increasing $p\text{CO}_2$, there are opposing views over the magnitude and even the direction (increase or decrease) to which this will occur, and it would appear that this may be dependent on community composition, hydrodynamic conditions or possibly even, the methodological procedures employed. Riebesell *et al.* (2007) have provided evidence for increased photosynthetic carbon fixation at elevated conditions of $p\text{CO}_2$ during mesocosm experiments in Norwegian fjords which resulted in positive changes in C:N stoichiometry. In contrast Joint *et al.* (pers. comm.) observed the opposite during similar experiments. Calcification by several groups of coccolithophores has been shown to be adversely effected by ocean acidification, though other workers have described how this effect varies between groups and observed increases in calcification and photosynthesis by the coccolithophore *Emiliania huxleyi* at elevated levels of $p\text{CO}_2$ (see Ziveri *et al.*, this volume). When considering the fundamental role of this process and its relevance to ocean productivity, nutrient balance, gas exchange and carbon export, it would seem that the great uncertainty over the impact of ocean acidification would put it at the fore of priority research areas in the Mediterranean.

Trace elements

Decreases in ocean pH theoretically increase the proportion of free dissolved trace metals, and increase metal solubility, which could in theory lead to substantial increases in the total bioavailable fraction of many trace elements, perhaps resulting in toxic concentrations. Micronutrients (Fe, Co, Zn, Cd, Cu, etc.) are essential components of numerous metalloenzymes, which ultimately regulate microbial activity, productivity and thus carbon export in the oceans. For example in the North Atlantic Ocean, Fe has been shown to co-limit N-fixation and limit C-fixation in natural populations. The intracellular regulation of Fe is fundamental for C-fixation (within photosystems I and II, cytochromes and ferridoxin), nitrogen assimilation (e.g. nitrate reductase) and nitrogen fixation (nitrogenase). Both Zn and Co are the essential metal cofactors for carbonic anhydrase which is also essential for C-fixation. Cobalt is also required in many species of *Prochlorococcus* and *Synechococcus cyanobacteria*, and laboratory culture experiments performed at PML, have suggested that Co and Zn may limit nitrogen fixation by the filamentous cyanobacterium *Trichodesmium*. Trace metal budgets of the western Mediterranean Sea suggest that the main input of dissolved trace metals is from the eastern Atlantic ocean through the Strait of Gibraltar, with atmospheric inputs constituting the second largest input at the basin scale and rivers playing a minor input (Elbaz-Poulichet *et al.*, 2001). While the variable sporadic supply of atmospheric Saharan dust is considered perhaps the most significant source of many essential metal nutrients such as Fe to the eastern Mediterranean (Statham and Hart, 2005), the present view is that Fe is highly unlikely to be limiting in the Mediterranean Seas (Statham and Hart, 2005). Thus changes or increases in the bioavailable fraction of many trace metals due to ocean acidification of the Mediterranean are more likely to result in changes in the composition of microbial assemblages and/or toxic effects; with more local regional variability in the manifestation and magnitude of these changes being of paramount importance.

IMPLICATIONS OCEAN ACIDIFICATION TO THE MEDITERRANEAN SEA

Following their observations of increased levels of $p\text{CO}_2$ on carbon and nitrogen fixation by *Trichodesmium*, Hutchins *et al.* (2007) stated that "many of our current concepts describing the interactions between oceanic nitrogen fixation, atmospheric CO_2 , nutrient biogeochemistry, and global climate may need re-evaluation to take into account these previously unrecognized feedback mechanisms between atmospheric composition and ocean biology." This sentiment is entirely relevant to all of the aforementioned processes, particularly with respect to the Mediterranean, and it would appear that there is an urgent need for co-ordinated experimental and modelling investigations to address these concerns.

Predicting the response of microbial and metazoan communities and ultimately the functioning of ecosystems to ocean acidification is at a very early stage (e.g. Blackford and Gilbert, 2007; Widdicombe and Spicer, 2008). High CO_2 and acidification affect a number of ocean processes including nitrification, denitrification and carbon fixation (Royal Society, 2005) and have also been shown to impact on nitrogen fixers in culture (Hutchins *et al.*, 2007). Currently held beliefs

though are not all in agreement and are often conflicting, so that the magnitude, and in some instances even the direction, of organism or process response to CO₂ enrichment is not robustly characterised.

There are a number of issues not addressed here that are relevant to this discussion and which include: 1) the potential of microbial organisms and their populations to acclimate or adapt to changes in pH, 2) the synergistic product of increasing temperatures with ocean acidification, 3) the possible changes to nutrient supply following increased stratification, and 4) changing toxicity or bioavailability of pollutants (e.g. heavy metals) on biogeochemical cycles.

In summary of the above discussion we propose a number of untested hypotheses concerning ocean acidification predictions which may impact on the biogeochemistry of the Mediterranean:

- *Hypothesis #1* - Enhanced oligotrophy: the combination of reduced nitrification with elevated denitrification in sediments will accelerate the removal of NO₃⁻ from overlying waters and enhance the oligotrophic nature of offshore waters.
- *Hypothesis #2* - Further P limitation: positive changes to the N:P stoichiometry may result from a shift in NH₄⁺:NH₃ equilibria, which will be exacerbated by increases in bacterial N fixation to ultimately drive the ecosystem further into P limiting conditions. This may be as function of, or enhanced by, an increase in the availability of trace metals which are essential for the activity of metalloenzymes.
- *Hypothesis #3* - Reduced productivity and carbon export: inhibition of nitrification may decrease the ratio of NO₃⁻: NH₄⁺. In surface waters this will impact on rates of new and regenerated production and ultimately may lower the rates of exportable carbon, with direct impacts on productivity (including fisheries) and sequestration to the deep ocean.

It is however evident that a number of these potential impacts may counter each other, or compound each other with a degree of synergy that cannot be described from experimental observations alone. Models currently provide the only mechanism by which we can interrogate the multi-faceted relationship between these processes in order to investigate future ecosystem function. With little robust information suitable for parameterising such models, there is an urgent requirement for co-ordinated experimental and model studies to address the issue of ocean acidification in the Mediterranean Sea.

Biogeochemical impacts of ocean acidification - emphasis on carbonate production and dissolution

Marion Gehlen

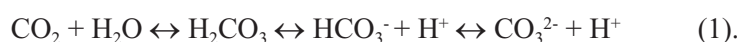
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ABSTRACT

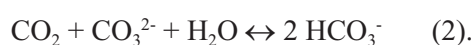
The awareness of impacts of ocean acidification on carbonate chemistry has revived research on the marine CaCO₃ cycle. Because it changes the saturation state of waters with respect to carbonate minerals, ocean acidification will impact on carbonate production and dissolution. Global biogeochemical ocean models provide a key to understanding and predicting the future of the marine carbonate cycle. I present a synthesis of published model studies on impacts of ocean acidification on pelagic carbonate production and the associated feedback to atmospheric CO₂. All models considered in this study compute bulk rates of the global carbonate cycle in line with recent observation-derived estimates. These models differ in degree of complexity and in the timeline of atmospheric CO₂ forcing. Despite these differences, the models predict a comparable response (decrease) of pelagic calcification for a given future CO₂ level compared to the preindustrial value, as well as a similar order of magnitude of the feedback associated with lower carbonate production. While state-of-the-art biogeochemical models have the necessary skills for projecting changes in carbonate chemistry in response to climate change and ocean acidification, they are at present not suitable for ecological impact assessment.

INTRODUCTION

Since the beginning of industrialization, mankind has emitted 361 Gt C to the atmosphere of which the ocean has absorbed approximately 155 Gt C (between 1800 and 1999, according to Sabine *et al.*, 2004), making the world ocean the largest sink of anthropogenic CO₂. The uptake of CO₂ by the ocean is primarily a physico-chemical process. As CO₂ penetrates seawater, it behaves like a weak acid dissociating according to



Reaction (1) results in an increase of [H⁺] and thus a drop in pH, a process referred to as ocean acidification (e.g. Caldeira and Wickett, 2003; Feely *et al.*, 2004; Orr *et al.*, 2005). Acidification of ocean water goes along with a decrease in carbonate ion concentration and of the saturation state with respect to carbonate minerals (CaCO₃). This can be represented as the titration of carbonate ions by carbonic acid:



The decrease in saturation state with respect to CaCO₃ directly impacts its formation and dissolution. In the modern marine environment, carbonate formation is largely a biotic process¹. Marine organisms form shells and skeletons of a variety of carbonate minerals: high-Mg calcite

¹ 'whitings' reported in the vicinity of the Great Bahama Bank are a notable exception (Morse *et al.*, 2003).

(coralline algae), aragonite (pteropods and corals, some foraminifera and bivalves), calcite (coccolithophores, foraminifera, some bivalves), as well as mixed-layered calcite and aragonite (certain bivalves). The solubility of a carbonate mineral depends on its composition and structure with solubility increasing from calcite over aragonite to high-Mg calcite. While it is straightforward to envision that these differences in solubility translate into a variable susceptibility to dissolution, one might also expect differing degrees of vulnerability of calcifying organisms to ocean acidification.

The reaction pathway of biocalcification varies between taxa and awaits further elucidation. The response of calcification to changes in seawater carbonate chemistry was assessed for a variety of organisms. These studies mostly reported a decrease in calcification with decreasing saturation state before undersaturation was reached (refer to Kleypas *et al.*, 2006) for a synthesis of existing experimental evidence up to 2005). Calcification rates were either linearly related to the saturation state (corals) or could be described by a threshold value of the saturation state below which the calcification decreased rapidly (foraminifera and coccolithophores) (e.g. Gattuso *et al.*, 1998; Bijma *et al.*, 1999; Kleypas *et al.*, 1999a; Riebesell *et al.*, 2000; Zondervan *et al.*, 2001; 2002). Recent studies suggested that the response of calcification to saturation state might be more variable than expected. For instance, Iglesias-Rodriguez *et al.* (2008) reported that the coccolithophore *Emiliania huxleyi* responded with increased calcification to a decrease in saturation state, much to the contrary of results published by Riebesell *et al.* (2000). The latter found a decrease in calcification. According to Langer *et al.* (2006), calcification as a function of $p\text{CO}_2$ (or saturation) is best described by an optimum curve centered at present day $p\text{CO}_2$ (± 360 ppm) for the coccolithophore *Calcidiscus leptoporus*. On the other hand, it appears not to be affected by $p\text{CO}_2$ increase in the case of *Coccolithus pelagicus*. The dependency of biocalcification on saturation state clearly warrants further attention.

The emerging diversity of responses of calcifiers to a decrease in saturation state of seawater challenges global ocean biogeochemical models. These models mostly represent CaCO_3 formation as a geochemical source/sink function assigned to a specific plankton size class. Despite their limitations, they are important tools for assessing changes in carbonate chemistry (e.g. Orr *et al.*, 2005) and deliver first order quantification of biogeochemical impacts and feedbacks to atmospheric CO_2 (e.g. Heinze, 2004; Ridgwell *et al.*, 2007; Gehlen *et al.*, 2007; Gangstø *et al.*, 2008). Decreasing CaCO_3 production and increasing dissolution in response to ocean acidification both would act as negative feedbacks to atmospheric CO_2 . While carbonate production induces a $p\text{CO}_2$ increase associated with precipitation, enhanced dissolution of CaCO_3 increases the alkalinity of ocean waters. A decline in CaCO_3 production, as well as enhanced dissolution will both favor the ocean uptake of CO_2 .

The feedback associated with changes in CaCO_3 production and dissolution should however not be considered in isolation. A decrease in calcification will alter the ratio of organic C to inorganic C (the rain ratio): an increase in rain ratio will promote the dissolution of CaCO_3 in marine sediments resulting in a higher alkalinity (Archer and Maier-Reimer, 1994), a negative feedback operating on timescales of 10,000 years. Alternatively, if the ratio of organic C to inorganic C is closely bound by the process of ballasting (e.g. Armstrong *et al.*, 2002; Klaas and Archer, 2002), less CaCO_3 production would imply less ballasting of organic C (POC) fluxes and a shallower remineralization of POC which corresponds to a positive feedback on atmospheric CO_2 .

I will present a critical overview of published estimates of changes in global marine CaCO_3 cycle under increasing atmospheric CO_2 . When possible the order of magnitude of associated feedbacks will be quoted. Global biogeochemical ocean models lack the spatial resolution for a realistic representation of shelf and coastal environments. The fate of shallow water CaCO_3 production (e.g. Mackenzie *et al.*, 2004; Andersson *et al.*, 2006) is thus excluded from this discussion.

THE GLOBAL MARINE CARBONATE CYCLE: RECENT ESTIMATES AND MODEL RESULTS

The awareness of important changes in the ocean C system in response to the uptake of anthropogenic CO_2 and the likely impacts of ocean acidification on carbonate production has revived research on the marine CaCO_3 cycle. The report published by Iglesias-Rodriguez *et al.* in 2002a was still largely based on the synthesis by Milliman and Droxler (1996). Recent budget

estimates suggest a higher CaCO_3 production and an increased importance of pelagic dissolution at shallow depths and thus above the saturation horizon with respect to the most common CaCO_3 forms, compared to older studies. In Table 1 observational based estimates are compared to output from models for which the corresponding information is available.

The comparison between modeled rates and observation based estimates suggest a good correspondence between both. Global bulk numbers of the marine CaCO_3 cycle are in general well reproduced by biogeochemical models (e.g. Heinze, 2004; Gehlen *et al.*, 2007; Ridgwell *et al.*, 2007; Gangstø *et al.*, 2008). A careful analysis of the partitioning of dissolution fluxes with depth reveals however that the models fail to capture the large dissolution fluxes occurring between 0 and 2,000 m depth (e.g. Feely *et al.*, 2004). The exact nature of processes behind high dissolution fluxes at shallow depth (and thus above the calcite and aragonite saturation horizon) inferred from the analysis of alkalinity fields and particle fluxes still awaits identification. It has to be noted, however, that with the exception of the study by Gangstø *et al.* (2008), models equate CaCO_3 to calcite. Gangstø *et al.* (2008) consider calcite and aragonite in their study. Their results suggest that these dissolution fluxes might reflect the contribution of CaCO_3 phases more soluble than calcite.

Table 1. The global CaCO_3 cycle: model output *versus* observations.

Units = Gt C/yr	Models		Observation-based estimates		
	Gehlen <i>et al.</i> , 2007	Gangstø <i>et al.</i> , 2008	Iglesias- Rodriguez <i>et al.</i> , 2002	Feely <i>et al.</i> , 2004	Berelson <i>et al.</i> , 2007
Euphotic CaCO_3 Production	1.3	1.1	0.7-1.4	0.8-1.4	0.5-1.6
Net (= export) CaCO_3 Production	0.8	0.9	1.1±0.2		0.4-1.8
Total pelagic CaCO_3 dissolution	0.5	0.5		0.5±0.2	1.0±0.2
% total CaCO_3 diss between 0-2,000m	34	58		≥ 60	

THE GLOBAL MARINE CARBONATE CYCLE UNDER HIGH ATMOSPHERIC CO_2

Table 2 provides an overview of published forecasts of the fate of marine CaCO_3 production in a high CO_2 world. These studies have been performed with models of varying complexity and spatial resolution. An in-depth comparison of the models is beyond the scope of this chapter. Even more important is the disparity of acidification scenarios selected and the associated time span. The approach taken by the authors for implementing a dependency of CaCO_3 production on seawater carbonate chemistry is yet another fundamental difference. However, all make use of the same limited number of experimental data sets. Heinze (2004) relied on Zondervan *et al.* (2001) to describe the dependency of calcification on atmospheric CO_2 as a function of the departure of modeled future surface ocean $p\text{CO}_2$ from pre-industrial values. Gehlen *et al.* (2007), as well as Gangstø *et al.* (2008), represented CaCO_3 production as a function of undersaturation by fitting a Michaelis-Menten equation through a combined data set obtained for coccolithophores from laboratory and mesocosm experiments. Ridgwell *et al.* (2007) chose to fit a higher order reaction kinetics to each individual published data set, thus including coccolithophores, foraminifera and corals. In the light of available experimental evidence on the response of calcifiers to changes in carbonate chemistry, each of these approaches is highly speculative. The model studies should be seen as biogeochemical sensitivity studies, rather than as attempts to quantify the biological impacts.

Heinze (2004) predicts a global decrease in CaCO_3 production of 50% at the end of his model experiments. The final atmospheric CO_2 concentration was 1,413 ppm (experiment A, Table 1), respectively 1,403 (experiment B, Table 1). This is higher than in Gehlen *et al.* (2007) (1,112 ppm, Table 1). At an atmospheric $p\text{CO}_2$ of 1,112 ppm CaCO_3 production declined by approximately 38% (Fig. 3 in Heinze, 2004) compared to 27% in Gehlen *et al.* (2007). This level of atmospheric $p\text{CO}_2$ was reached after 140 years in Gehlen *et al.* (2007) compared to 420 years in Heinze's study. The longer duration of the latter experiment contributes to the greater amplitude in calcification response. Similarly, Ridgwell *et al.* (2007) conclude that despite differences between models and scenarios, the projected decrease in pelagic calcification and associated increase in atmospheric

CO₂ uptake by the ocean over the next century, converge. Needless to say, CaCO₃ dissolution is included as a function of undersaturation in all models and the effect of changes in the buffer capacity of ocean waters factor is taken into account.

Table 2. Synthesis of published results on the future of CaCO₃ production in a high CO₂ world.

CO ₂ forcing scenario	Exp. ID	Net (= export) CaCO ₃ prod. (Gt C/yr)	ΔCaCO ₃ prod. (%)	Feedback (ppm)	Reference
Hist+A1B+stabilization up to 2250	A	0.8	-50	10	Heinze, 2004
	B	1.6	-50	20	
Hist+IS92a+linear decrease 2100-2300+ stabilization 2300-3000	all	1.05-1.59	-	18-49	Ridgwell <i>et al.</i> , 2007
	mean	1.3	-54 (in 2100)	-	
278 to 1,112 ppm; 1% increase per year		0.8	-27	3	Gehlen <i>et al.</i> , 2007
SRES20C3M, 1861-1999 + SRES A2, 2000-2100)		0.9 (total)	-19		Gangstø <i>et al.</i> , 2008
		0.6 (calcite)	-13	n.a.	
		0.3 (aragonite)	-29		

DISCUSSION

State-of-the-art ocean biogeochemical models have the capacity to project changes in ocean carbonate chemistry in response to climate change and ocean acidification (e.g. Orr *et al.*, 2005). Model studies published up to now agree in attributing a weak negative feedback on century timescales to atmospheric CO₂ associated with the combined effect of decreasing CaCO₃ production and increasing dissolution. This negative feedback might in part be offset by changes in the penetration depth of particulate C due to less ballasting of POC fluxes by CaCO₃. The consequences of ocean acidification on the efficiency of the soft tissue pump are poorly understood and it cannot be excluded that the positive feedback related to a shoaling of the penetration depth of POC will override the feedback associated with decreased CaCO₃ production.

In a world where CaCO₃ production would be largely an abiotic process, the model studies discussed so far would provide a robust quantification of impacts and feedbacks. This is clearly not the case in the modern ocean where CaCO₃ production equates with biocalcification. The diversity of the response of marine calcifiers to changing CaCO₃ chemistry puts our capability to model marine ecosystems to the test. In order to comprehend the consequences of unmitigated ocean acidification we have to move beyond biogeochemistry towards ecology. One way of achieving progress would be to combine projections of the future ocean and models that predict the geographic distribution of calcifiers in response to environmental forcing (e.g. Iglesias-Rodriguez *et al.*, 2002b). The development of models with an increasing number of plankton functional types is another answer to the challenge of forecasting future changes in marine ecosystems (Le Quéré *et al.*, 2005). Despite their increased complexity, these models are based on the same fundamental principles than those discussed above. They are likely to suffer from the same limitations when it comes to representing the diversity of biological responses to ocean acidification. The consideration of the coexistence of different ecotypes sharing a common biogeochemical functionality appears as a promising alternative. In all these cases, model development will be tributary to advances in our understanding of the organism and community response to ocean acidification.

Acknowledgments: this work is a contribution to the project “Marine Carbon Sources and Sinks Assessment” (CarboOcean) funded under the European Community’s Sixth Framework Programme and the “European Project on Ocean Acidification” (EPOCA) which received funding from the European Community’s Seventh Framework Programme.

High-resolution geochemical records from Mediterranean cold-water corals: proxies for paleoclimate and paleoenvironmental reconstructions and the role of coral physiology

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ABSTRACT

The chemical composition of the skeletal hard parts of the azooxanthellate scleractinian coral *Desmophyllum dianthus* retrieved from various sites in the Mediterranean Basin was analysed by laser ablation ICP-MS in order to investigate the potential of this species as paleoenvironmental and paleoclimate archive and assess the organism's physiology control on the chemical signal. The coral calcification is a biologically-mediated process which can induce large geochemical offset from the expected equilibrium values and this is particularly evident once the analytical spatial resolution is increased. Our data suggest that the majority of the elements analysed (B/Ca, Mg/Ca, Sr/Ca, U/Ca, P/Ca) show a strong spatial heterogeneity linked to the coral microstructure (centres of calcification vs. fibrous aragonite), with some of the elements varying by more than a factor of four.

Given the near constant environment in which azooxanthellate corals live, factors other than temperature, such as the different precipitation rate and/or the different organic content between COCs and FA, might be responsible for the geochemical heterogeneity. The skeletal portion close to the outer septal surface displays low variability of the geochemical signal and so it is considered the best candidate for paleoenvironmental reconstructions. Therefore, the possibility to retrieve reliable paleoenvironmental information from *D. dianthus* can be achieved by a selective sampling of specific ultra-structures, in order to minimise 'vital effect', non-carbonatic contaminations, and reduce noise on paleoclimate and paleoenvironmental signals.

INTRODUCTION

The possibility to retrieve high-resolution paleoclimate and paleoceanographic data from the aragonite exoskeleton of scleractinian azooxanthellate corals has recently become a topic of increasing research interest and is achieved mainly by evaluating their stable isotopes, trace and minor element compositions (Adkins *et al.*, 1998; Eltgroth *et al.*, 2006; Cohen *et al.*, 2006; Rüggeberg *et al.*, 2008; Montagna *et al.*, 2006).

Cold-water corals are among the most promising archives for intermediate and bathyal water depths, providing higher resolution (although more discontinuous with time) than sediment cores (e.g. sub-decadal vs. 100-500 years for sediments) and not being affected by bioturbation. Moreover, cold-water corals can be radiometrically dated using both high precision U-series and ^{14}C methods. By coupling radiocarbon data with P/Ca ratios, which has been recently demonstrated to be a proxy for nutrients in azooxanthellate corals (Montagna *et al.*, 2006), from the same absolutely dated sample (U/Th), it will be possible to calculate the ocean ventilation rates, which have an important control on the atmospheric CO_2 . P/Ca micro-analyses will also allow us to quantify the fluxes of nutrients to intermediate and deepwater environments and the past ocean productivity, furthering our understanding on the biological functions of the ocean in regulating atmospheric CO_2 . The Mediterranean Sea plays a role in CO_2 sequestration via the transfer of carbon from the surface water to the intermediate Atlantic waters across the Strait of Gibraltar, and the understanding of this process in the past is crucial for future CO_2 sequestration predictions. Although corals have been shown to be formidable archives of environmental change, offering a continuous and often undisturbed record, coral physiology has to varying degrees imprinted a 'vital effect' during skeletogenesis, complicating the interpretation of climatic records. Trace elements and stable isotopes are often correlated with the coral micro- and ultra-structures (i.e. centres of calcification and fibrous aragonite), suggesting a strong control of biological processes on skeleton composition, which may distort environmental signal (Sinclair *et al.*, 2006; Meibom *et al.*, 2007). Recently, Blamart *et al.* (2007), studying the coral *Lophelia pertusa* for B isotopic composition, which is believed to be a marine paleo- $p\text{CO}_2$ proxy in biogenic carbonate (Hönisch *et al.*, 2004; Pelejero *et al.*, 2005), reported a clear correlation between $\delta^{11}\text{B}$ and the skeletal ultra-structure, suggesting caution in using this geochemical proxy. To date, the skeletogenesis process in azooxanthellate corals is largely unknown and the fine-scale geochemical composition has been poorly explored due to analytical limitations.

Confidence in paleo-climate reconstructions consequently requires an in deep understanding of the absolute magnitude and pattern of 'vital effect' upon different architectural elements, their spatial heterogeneities and the use of proper analytical methods with increasing fine-scale resolution. We investigated minor and trace element variations across skeletal features of both living and fossil specimens of *Desmophyllum dianthus* collected in various oceanographic settings in the Mediterranean Basin (Figure 1). The mid-plane of S1 septa (the largest septum) (Figure 2) has been targeted for B/Ca, Mg/Ca, Sr/Ca, U/Ca and P/Ca ratios using the pulsed laser ablation system connected to a Varian 820 MS at the Research School of Earth Sciences, ANU (laser ablation analytical procedure, data processing, the overall precision and long-term reproducibility have been described by Montagna *et al.*, 2006; 2007). Here we report the minor and trace element composition of one of the fossil samples (sampling location: white dot in Figure 1), which is representative of the geochemical patterns of all the other living and fossil samples analysed. The aim of this paper is to provide a picture of the geochemical heterogeneity of the cold-water coral *D. dianthus* across different skeletal components, assessing the best coral portion to be analysed in order to obtain reliable paleoenvironmental and paleoclimate reconstructions not distorted by the coral physiology.

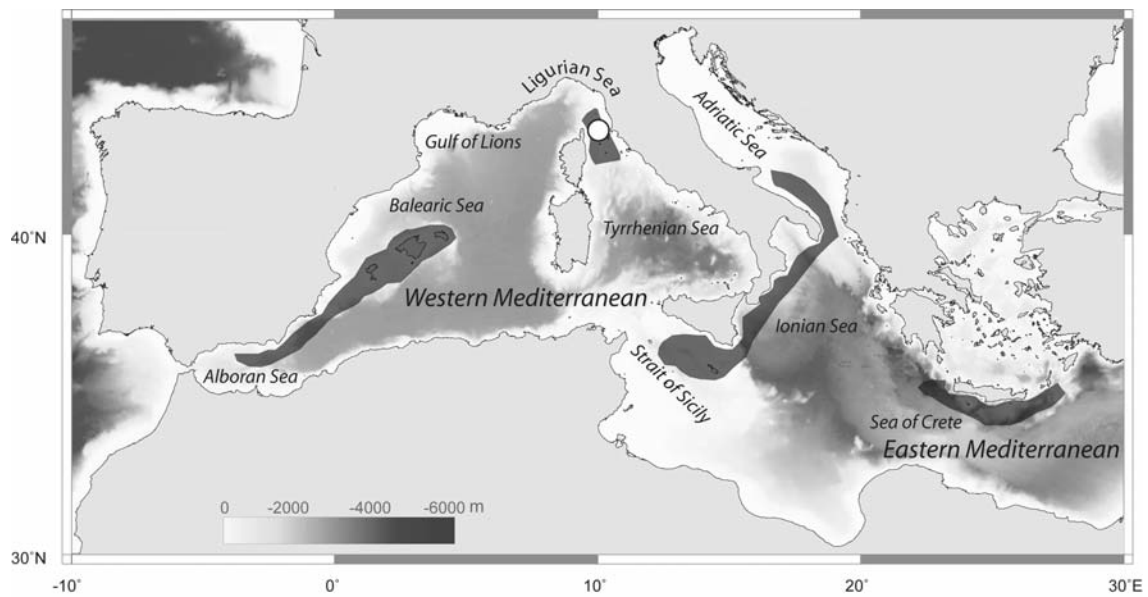


Figure 1. Map of the Mediterranean Sea with the coral sampling locations (dark-grey areas). The white dot represents the azooxanthellate coral *Desmophyllum dianthus* discussed in the text.

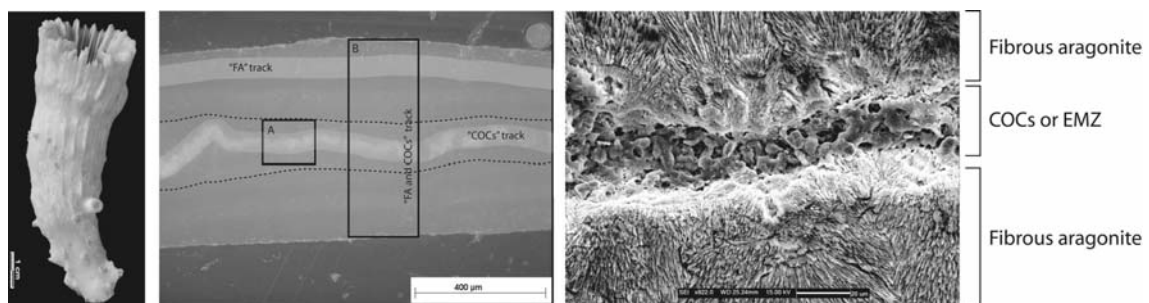


Figure 2. Left: *Desmophyllum dianthus* in lateral view showing the well-developed radially symmetric S1 septa. Middle and right: reflected light microscope and SEM images of a transversally sectioned S1 septum, showing rounded aggregates of aragonite crystals surrounded by bundles of fibrous aragonite. SEM image is an enlargement of the inner septal portion (A). Dashed lines represent a distinct border between deposits of centre of rapid accretion (*sensu* Stolarki, 2003) and thickening deposits. The laser ablation transects along the fibrous aragonite portion and the centres of calcification were obtained using a 80 μ m diameter spot following the growth direction (light grey lines). Rectangle B represents the laser ablation (20x220 μ m slit) track crossing the FA and COCs.

RESULTS AND DISCUSSION

Figure 2 shows the pictures of the coral *D. dianthus* (sample LM99-124) and the twofold microstructure typical for most of the zooxanthellate and azooxanthellate corals, with the central septal portion composed of an aggregation of μ m-sized granular crystals, surrounded by bundles of acicular aragonite perpendicular to the wavy line of the centres of calcification (COCs). A distinct region exists in the middle portion of the septum, which is similar to the “deposits of Center of Rapid Accretion” described by Stolarki (2003), beyond which fibrous aragonite (FA) is organized into growth layers. COCs and FA are also distinctly different in terms of minor and trace elements (Figure 3, Table 1), as previously reported by Meibom *et al.* (2006) for the zooxanthellate coral *Colpophyllia* sp., Sinclair *et al.* (2006) and Cohen *et al.* (2006) for *Lophelia pertusa* and Robinson *et al.* (2006) and Gagnon *et al.* (2007) for *Desmophyllum dianthus*. In particular, data in Figure 3, extending the results by Robinson *et al.* (2006) and Gagnon *et al.* (2007) to other elements, such as B/Ca and P/Ca, confirm that vital effect is active at micron-scale length, affecting the partitioning of most of the elements.

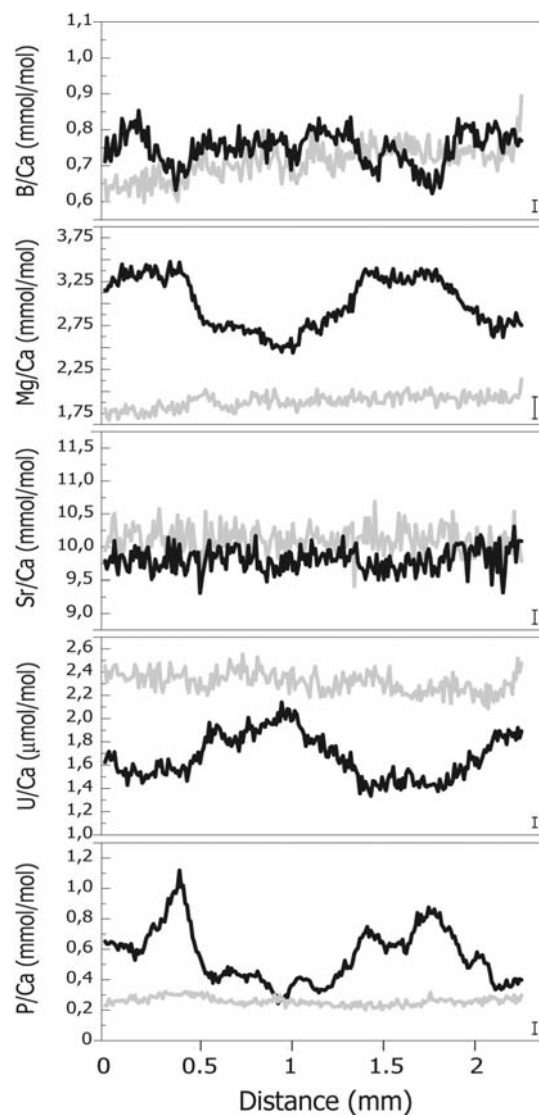


Figure 3. B/Ca, Mg/Ca, Sr/Ca, U/Ca and P/Ca ratios measured along two laser ablation tracks on the transverse sections of the S1 septum. Black and grey lines represent the laser ablation tracks across the centres of calcification (COCs) and the fibrous aragonite (FA), respectively.

Table 1. Distribution of B/Ca, Mg/Ca, Sr/Ca, U/Ca and P/Ca ratios between COCs and FA.

Coral Microstructure	Element	Distribution	Coral Microstructure
Centre of calcification or Early Mineralization Zone	B/Ca	≥	Fibrous Aragonite
	Mg/Ca	>	
	Sr/Ca	<	
	U/Ca	<	
	P/Ca	>	

The region of the COCs is depleted in U/Ca and Sr/Ca and enriched in Mg/Ca and P/Ca and to a lesser degree B/Ca compared to the FA (Figures 3 and 4). In particular, the U/Ca and Sr/Ca variation is ~ 90% and 10%, respectively, Mg/Ca doubles in the COCs and P/Ca vary even more significantly by a factor of ~ 4.

The COCs exhibit the greater geochemical variability, with Mg/Ca, U/Ca and P/Ca varying by ~ 40%, 50% and 450%, respectively, across the entire transect. This is very likely related to the

dimension of the laser beam, which produces an 80 μ m-diameter spot, too large to target only the COCs and therefore incorporating different proportions of other coral microstructures. Values corresponding to ~ 3.4 mmol/mol for Mg/Ca and ~ 1.4 μ mol/mol for U/Ca represent the best estimate for the composition of pure COCs, whereas lower (for Mg/Ca) and higher (for U/Ca) values, respectively, reflect different mixing ratios between COCs and FA, the two compositional end-members contributing to the geochemical composition of the coral septum.

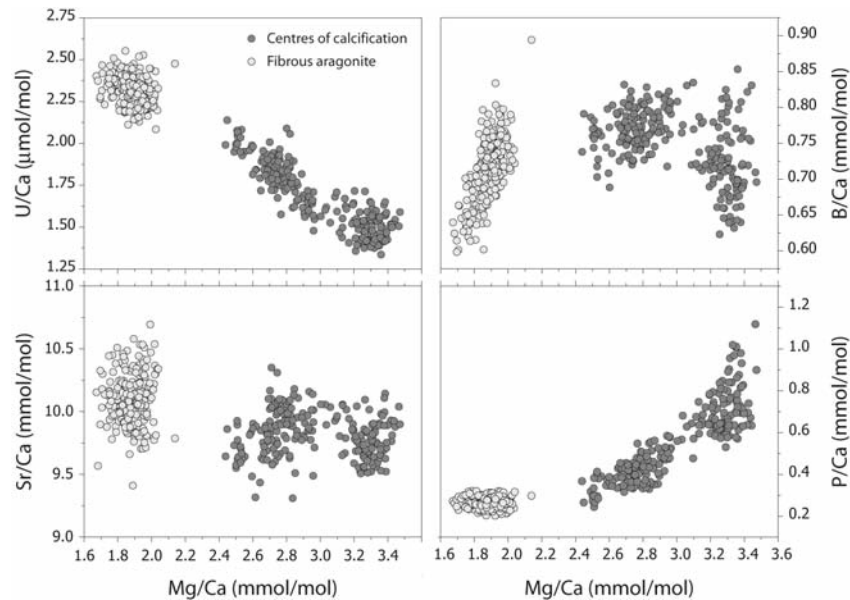


Figure 4. Mg/Ca vs. U/Ca, Sr/Ca, B/Ca and P/Ca ratios. Centres of calcification are significantly enriched in Mg/Ca and P/Ca and depleted in U/Ca compared to the fibrous aragonite.

The portions close to the outer septal surface are primarily composed of FA, which forms the “thickening deposits” described by Stolarski (2003); therefore an 80 μ m-diameter spot is small enough to target only this specific microstructure. Accordingly, the elemental composition of this coral portion is less variable with changes by less than 20% for Mg/Ca and U/Ca and 40% for P/Ca. Considering this laser ablation track as representative for the FA, the values of ~ 1.8 mmol/mol for Mg/Ca and ~ 2.3 μ mol/mol for U/Ca are representative of the FA end-member. The above values are confirmed by analyzing the septum perpendicularly to the growth direction and crossing both the FA and COCs (Figures 2 and 5). Mg/Ca and U/Ca values steeply increase and decrease, respectively, within the central band (coral portion between the two dashed lines in Figure 2), displaying a pattern with a similar shape (Figure 5).

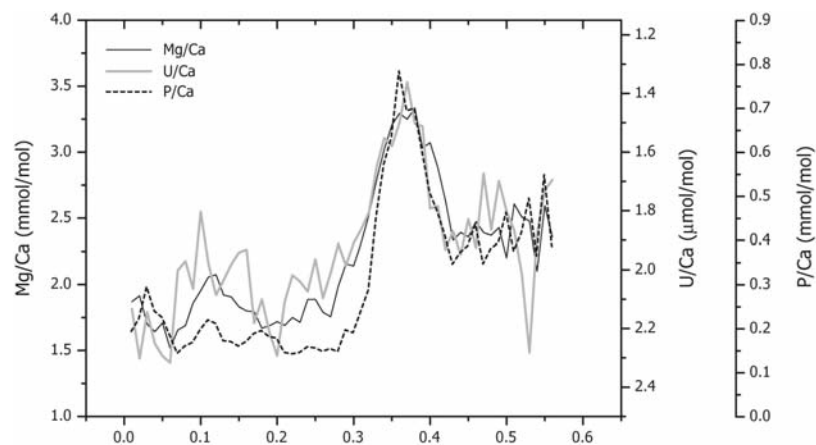


Figure 5. Mg/Ca, U/Ca and P/Ca ratios measured along the transect perpendicular to the growth direction and crossing both the FA and COCs (see rectangle B in Figure 2). Note that y-scale for U/Ca is inverted.

A similar two-fold increase in Mg/Ca in COCs compared to FA was previously reported by Gagnon *et al.* (2007) for *D. dianthus* and represents a common feature in other scleractinian corals (Meibom *et al.*, 2006; Sinclair *et al.*, 2006). The enrichment of Mg/Ca ratios in the centres of calcification has been explained by Gagnon *et al.* (2007) as a result of the surface entrapment assuming rapid precipitation in this specific coral microstructure and/or the presence of organic material. Stolarski (2003) reported a much higher content of organic-enriched components in COCs with respect to the thickening deposit region (i.e. FA); this difference might be responsible for enriched Mg/Ca.

The significant negative correlation between Mg/Ca and U/Ca in the COCs analysed in the present study is indicative that the latter element is not controlled by variation in crystal growth rate and might be linked to other factors, such as pH variations. As also reported by Gabitov *et al.* (2008), the Mg/Ca and U/Ca partition coefficients in abiogenic precipitated aragonite display a strong positive growth rate dependence. Accordingly, the two elements should be positively, not negatively, correlated. Consequently, our results support the hypothesis of a pH control on uranium uptake in the coral microstructures, consistent with a decrease of the aqueous species (the species incorporated into aragonite; Reeder *et al.*, 2000) with increasing pH in the calcifying fluid.

If this hypothesis is correct, the similar shape of the patterns for Mg/Ca and U/Ca across the septum (Figure 5) implies that variations of crystal growth rate and pH are coupled. The coral organism is able to pump protons away from the calcifying space, elevating the pH and enhancing aragonite precipitation rates (Al-Horani *et al.*, 2003). A higher pH value in the rapidly calcifying centres of calcification has been predicted by geochemical models (Adkins *et al.*, 2003; Rollion-Bard *et al.*, 2003) and, consequently, a decrease in U/Ca ratios in this coral microstructure corresponds to an increase in Mg/Ca ratios. The reason of the higher P/Ca content in COCs compared to FA is presently unknown but one explanation is likely the presence of a higher content of P-rich organic matter or the occurrence of other mineral phases, such as crystalline hydroxylapatite inclusions (Mason *et al.*, 2007).

While a detailed knowledge of the elemental uptake mechanisms operating in the different skeletal components is still lacking, some conclusions can be drawn on the basis of the present results.

The most evident problem is that of the deconvolution between environmental signals encoded in the coral aragonite and the biological factors overprinting those signals.

The possibility to extract reproducible results that have environmental significance requires caution and a broad systematic analytical work at very fine resolution, which allows for a selective sampling strategy. Moreover, calibrations should be performed on specific ultra-structures in order to minimise 'vital effect' and reduce noise on paleo-climate and paleo-environmental reconstructions. This is the case for P/Ca, U/Ca and Mg/Ca ratios, which are particularly prone to microstructural variations. In particular, it seems that sampling FA along the cross-section of the septum should yield the most reliable paleo-climate and paleo-environmental reconstructions.

Species where different microstructures are clearly separated (i.e. centres of calcification vs. fibrous aragonite) should be selected as potential natural archives, whereas in other species data variability might be partially caused by a mixture of various proportions of different ultra-structures at a scale lower than analytical spatial resolution.

Loss of Mediterranean marine biodiversity in a high-CO₂ world

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SHORT ABSTRACT

We studied the effect of ocean acidification on Mediterranean marine biodiversity at volcanic carbon dioxide (CO₂) vent systems that had caused long-term gradients in seawater carbonate chemistry. Our observations verify concerns, based on model predictions and short-term laboratory experiments, that ocean acidification will likely cause a decrease in Mediterranean marine biodiversity and lead to shifts in ecosystem structure. CO₂ vents appear as valid natural laboratories that should be used to improve our scarce knowledge on the effect of ocean acidification on marine biota.

‘Ocean acidification’ describes the ongoing global decrease in seawater pH caused by dissolution of anthropogenic CO₂ from the atmosphere into the oceans and is one of the most serious threats facing marine life this century (Caldeira and Wickett, 2003). Atmospheric CO₂ increased by 32% between 1880-2005 (from 280 to 379 μatm) and is expected to double relative to preindustrial levels by 2100 (IPCC, 2007). Present-day CO₂ emissions are *ca* 9 GT C y⁻¹, over 25% of which enter the oceans (Canadell *et al.*, 2007), causing them to acidify due to the following reaction:



Major effects of this acidification include an increase in the amount of bicarbonate ions and a decline in the amount of carbonate ions. The latter lowers the saturation states of calcite and aragonite which are the chemical building-blocks of shells for a wide range of ecologically important organisms (e.g. coccolithophores, coralline algae, corals, molluscs and echinoderms).

Short-term laboratory experiments show that a decline in carbonate ions makes it more difficult for such organisms to produce and maintain their shells, raising widespread concerns about the future of our oceans in a high CO₂ world (Royal Society, 2005; Kleypas *et al.*, 2006). Mesocosm research has led to estimates that tropical coralline algal and coral reef calcification will cease below $\Omega_{\text{aragonite}} 3.3$, causing a shift toward communities dominated by non-calcified organisms at 480 ppm *p*CO₂ (Hoegh-Guldberg *et al.*, 2007; Kuffner *et al.*, 2008). The effects of experimental seawater acidification have mostly been measured over timescales of hours to months and the long-term effects of exposure to lowered pH levels were previously unknown. Some studies have added acid, calcium chloride or sodium bicarbonate to experimental tanks to examine the effects of lowered seawater pH (e.g. Gattuso *et al.*, 1998; Marubini and Thake, 1999; Marubini and Atkinson, 1999; Marubini *et al.*, 2003; Jokiel *et al.*, 2008). However, this approach does not alter Dissolved

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Inorganic Carbon (DIC) levels, which affect photosynthesis and calcification (Leclerq *et al.*, 2000; Langer *et al.*, 2006; Palacios and Zimmerman, 2007), so it is advantageous to elevate CO₂ levels to mimic the effects of ocean acidification on seawater carbonate chemistry (Iglesias-Rodriguez *et al.*, 2008). Mesocosm studies (Langdon *et al.*, 2000; Riebesell, 2004; Delille *et al.*, 2005; Widdicombe and Needham, 2007) and the Free Ocean CO₂ Enrichment (FOCE) approach¹ have been hindered by the difficulties of imitating acidified conditions *in situ* for sufficient periods to affect natural communities.

We are studying sites where shallow (0-10 m depth) volcanic CO₂ vents have marine communities that are tolerant of long-term elevations in *p*CO₂ (Figure 1) and so are well-suited to ocean acidification studies (Riebesell, 2008). The vent gases are 95% CO₂ and lack the toxic sulphur compounds that characterise the majority of Mediterranean vents (Dando *et al.*, 1999). The vents acidify seawater on sufficiently large spatio- and temporal scales to integrate ecosystem processes such as production, competition and predation. They also acidify intertidal rocky habitats, infralittoral rock, infralittoral sediment, cave-dwelling communities and *Posidonia oceanica* meadow (Figure 1), allowing investigations into the consequences of acidification on a range of different biotopes.

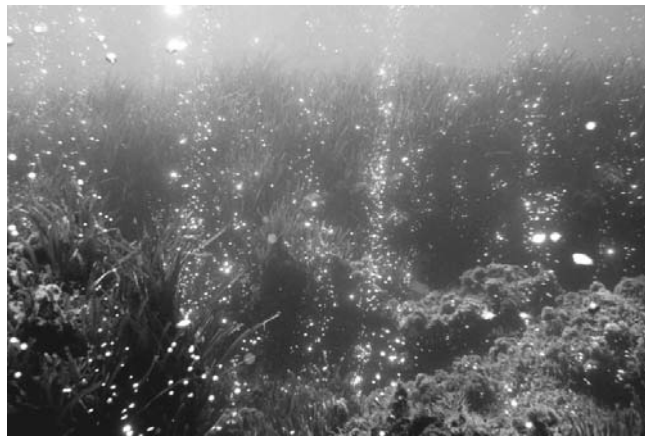


Figure 1. Shallow water CO₂ vents off Ischia (Gulf of Naples, Italy) in a *Posidonia oceanica* meadow, April 2007.

Since April 2007 we have been monitoring gas flow rate and ambient gradients in seawater pH, total alkalinity, salinity, dissolved oxygen and temperature to calculate calcium and aragonite saturation states. We have done this in various weather conditions to assess variability due to rainfall and wind-driven water exchange. We have quantified the abundance of dominant macroorganisms in intertidal and subtidal zones within and adjacent to the vents. We have also studied photosynthetic efficiency, growth dynamics, shoot density and epibiont calcium carbonate on *Posidonia oceanica* along a pH gradient. Preliminary results show that long-term exposure to high *p*CO₂ has dramatic impacts on calcifying organisms but that a suite of organisms are resilient and may benefit from these conditions (Hall-Spencer *et al.*, 2008; Martin *et al.*, 2008b; Rodolfo-Metalpa and Hall-Spencer, 2008). Along gradients of normal pH (8.1-8.2) to lowered pH (mean 7.8), typical rocky shore communities with abundant calcareous organisms shifted to communities lacking calcifiers (Figure 2). This shift in the community composition showed no indication of adaptation or replacement of sensitive species by others capable of filling the same ecological niche and confirms the need for concern about the future ecology of our seas in a high-CO₂ world (Hoegh-Guldberg *et al.*, 2007). Below a tipping point of mean pH 7.8, subtidal calcifiers such as coralline algae, corals and sea urchins were absent whereas intertidal calcifiers (e.g. snails, limpets and barnacles) were more resilient. Differences between species are likely due to their calcium carbonate structures, mainly composed by three forms:

1) high-Magnesian calcite is the most soluble and forms the skeleton of coralline algae and certain echinoderms;

¹ (<http://www.mbari.org/rd/projects/2008/initiatives/FOCE_08.html>)

- 2) aragonite is also highly soluble at lowered seawater pH and forms scleractinian skeletons and pteropod shells; and
- 3) calcite is the most resistant form of carbonate to dissolution and forms foraminiferan tests and coccoliths.

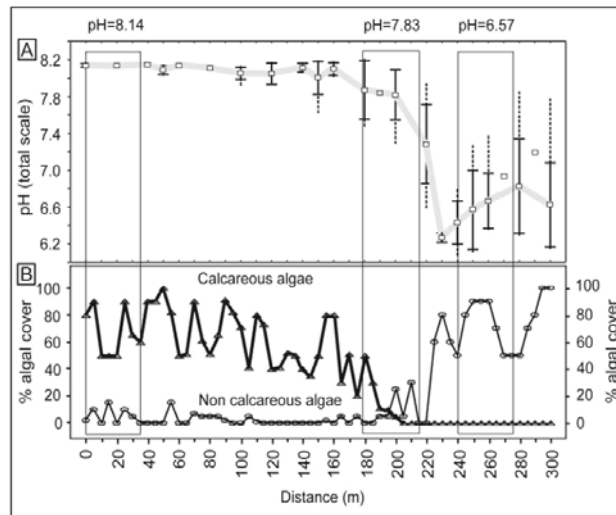


Figure 2. Mean (\pm s.d.) and total pH range (A) and the percentage cover (B) of calcareous algae (triangles) and non-calcareous algae (circle) along the vents, from normal pH (mean 8.14) to low pH (mean 6.57), April 2007.

Gastropods found at low pH appeared to be attracted to the vent areas by the abundance of algae but their shells were weakened (Figure 3A) and juvenile gastropods were absent below mean pH 7.7, presumably because their delicate shells were easily dissolved during recruitment. Corals, such as *Cladocora caespitosa* and *Balanophyllia europaea*, were absent around the vents and showed dramatic skeleton dissolution when transplanted to mean pH <7.8 (Figure 3B).

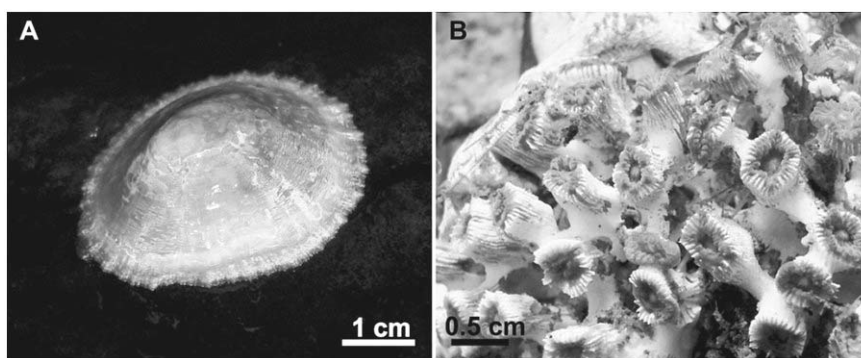


Figure 3. A) Live *Patella caerulea* collected at pH 7.4 showing shell dissolution; B) a heavily dissolved colony of the symbiotic coral *Cladocora caespitosa* maintained for six months at pH 7.4, June 2008.

In contrast, a suite of fleshy algal genera were resilient to low pH (e.g. *Caulerpa*, *Cladophora*, *Asparagopsis*, *Dictyota* and *Sargassum*). Some of these genera include invasive alien species which have begun to alter shallow marine ecosystems worldwide (Boudouresque and Verlaque, 2002). Seagrass thrived at low pH and lacked coralline algae that dominated the epiphytic community outside the vent areas. These corallines are important for the sediment budget of inshore areas and provide attachment for turf algae, sponges, foraminifera and polychaetes. A decrease in coralline

cover due to ocean acidification is expected to reduce seagrass biodiversity and alter associated food webs (Martin *et al.*, 2008b).

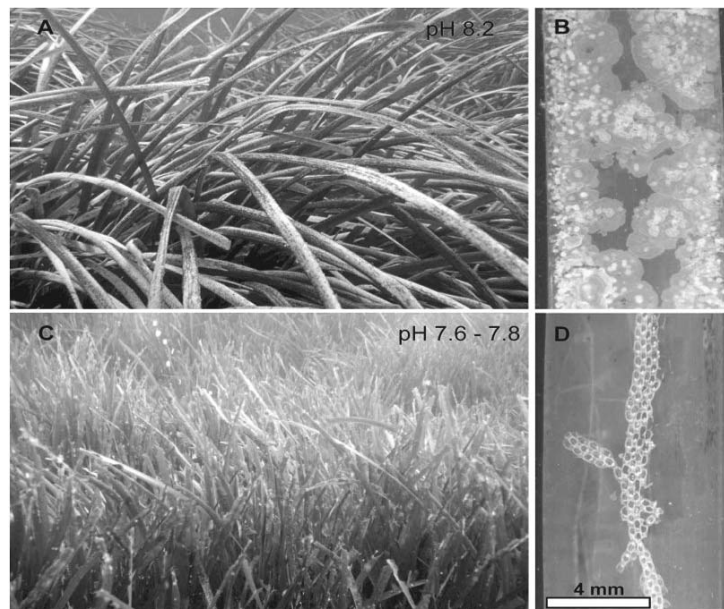


Figure 4. *Posidonia oceanica* at normal pH (A, B) showing abundant encrusting calcareous epibionts, and at low pH (C, D) without Corallinaceae April 2007.

In conclusion, we have begun to demonstrate that volcanic vents offer a useful tool for investigations into the long-term effects of increased seawater CO₂. Such sites are not perfect predictors of the effects of acidification as each is only a few 100 m² in extent and has variable pH levels. Fish, for example, can feed within the vent areas then seek refuge in surrounding normal pH water, an option that will clearly be unavailable in a globally acidified ocean. However our observations do add to concerns, based on laboratory experiments and model predictions, that ocean acidification will decrease marine biodiversity. We also show that Mediterranean vent systems provide a glimpse into the future for marine food webs and the functioning of coastal ecosystems if human CO₂ emissions continue unabated.

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Mediterranean corals under global warming and ocean acidification

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ABSTRACT

Anthropogenic-driven accumulation of CO₂ in the atmosphere and projected ocean acidification has raised concerns regarding the eventual impact on coral reefs. Little is known however about the physiological response of corals to increased *p*CO₂ and ocean acidification and it is difficult hence to predict what shifts these ecosystems will experience. It has been demonstrated that skeleton-producing corals grown in experimental acidified conditions are able to sustain basic life functions in a sea anemone-like form and will resume skeleton-building when reintroduced to normal modern marine conditions. However, in a natural environment and under ocean acidification conditions, calcifying organisms were absent. This can be attributed probably to ecological processes rather than physiological processes that drive shifts in benthic community structure. It should be noted that ocean acidification does not operate in isolation and that other stressors such as sea water elevated temperature and local stressors may act sooner than expected on coral communities, maybe before ocean acidification will affect these populations. The combination of global warming, ocean acidification and anthropogenic local stressors might shift coral populations to extinction within a few decades as is already apparent from some populations such as *Cladocora caespitosa* which reached extinction in some sites in the eastern Mediterranean Sea.

BACKGROUND

Since the beginning of the industrial era, massive burning of fossil fuels combined with deforestation have caused a rapid rise of atmospheric CO₂ levels, almost by two orders of magnitude faster than Earth has experienced in hundreds of millions of years. At present, the average concentration of atmospheric carbon dioxide stands on 387 ppmv, 37% more than 250 years ago (Harley *et al.*, 2006; Solomon *et al.*, 2007). However, CO₂ emissions do not cause a linear increase in the atmosphere since about a third of these outputs are being absorbed by the oceans, making the seas a significant buffer to anthropogenic combustions (Sabine *et al.*, 2004). Nevertheless, according to the Intergovernmental Panel on Climate Change (IPCC) atmospheric CO₂ concentrations are predicted to double by the end of this century if extreme measures will not take place.

CO₂ absorbed by the oceans is changing the water chemistry, especially within the photic zone where the vast majority of marine life and biological processes such as photosynthesis occur (Feely *et al.*, 2004; Orr *et al.*, 2005). This change is being expressed in an increase in bicarbonate

concentration in exchange to a reduction in carbonate concentration, which causes a decrease in calcium carbonate saturation state and in omega aragonite and calcite values, although differences in severity vary depending on altitude and region (Kleypas *et al.*, 1999a; Hoegh-Guldberg *et al.*, 2007). These changes lead to lowered pH. In fact the observed reduction of seawater pH from ~8.2 to ~8.1 over the last 100 years represents an increase of ~30% of H⁺ (Royal Society, 2005). Worst case scenarios predict that by the year 2300 oceanic surface water will be undersaturated for both calcite and aragonite (Caldeira and Wickett, 2003). Experimental evidence indicates that these changes will have an enormous effect on calcifying organisms such as scleractinian corals which usually show a decrease in calcification rate at reduced pH and low aragonite saturation state (Feely *et al.*, 2004; Schneider and Erez, 2006). While increase in carbon dioxide and bicarbonate can be beneficial for biological processes like photosynthesis where these ions are limiting sources, (Zimmerman *et al.*, 1997; Short and Neckles, 1999; Invers *et al.*, 2001), the concomitant decrease in carbonate availability can limit biological processes, such as calcification in many calcifying organisms in which carbonate concentration is the bottleneck, (Kleypas *et al.*, 1999b; Langdon and Atkinson, 2005; Orr *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007).

Scleractinian corals have aragonite skeleton which is the more soluble form of CaCO₃. They are more susceptible thereby to changes in carbonate saturation state and will be the first to be effected by its decrease (Guinotte *et al.*, 2003).

In recent years, with growing awareness to the change in ocean chemistry, numerous studies on the influence of seawater pH on different stony corals' species were conducted, mostly indicating a decrease in growth rates (Langdon *et al.*, 2000; Langdon and Atkinson, 2005). Deep water corals occur below 50 m (Cairns, 2007) and are perhaps the most vulnerable marine community to ocean acidification (Guinotte *et al.*, 2003). The 706 species of deep sea corals lack photosynthetic symbiotic algae (Turley *et al.*, 2007), at least six species are known to form substantial deep-water reef frameworks, creating biodiversity hotspots by providing structural habitat for other deep water organisms.

PRESENT STATE OF SHALLOW WATER CORALS OF THE MEDITERRANEAN SEA

There are over 40 species of scleractinian corals in the Mediterranean Sea of which only four are zooxanthellate. Out of these four symbiotic corals, one is considered a hermaphroditic species (*Cladocora caespitosa*), two aposymbiotic species (*Madracis pharencis* and *Oculina patagonica*), and one solitary coral (*Balanophyllia europea*). *C. caespitosa* (Linnaeus 1767) is the most important constructional species, which can form structures comparable to tropical reefs (Kružić and Benkovic, 2008).

Several informal reports suggest a decrease in the population size and spatial distribution of this species in the Mediterranean. Possible causes of this decrease may be global processes such as global warming and acidification, or more local causes such as competition with invading species, mainly algae (Kružić *et al.*, 2008) and invertebrates. Considering the better situation of *C. caespitosa* along the northern Mediterranean basins (Adriatic and Liguria), as compared with the deteriorating population along the Levant coast, suggests that temperature thresholds of the species are being crossed. This is also supported by reports of *C. caespitosa* mortality in the north-western Mediterranean under events of elevated sea temperature (Rodolfo-Metalpa *et al.*, 2005). Thirty years ago, *C. caespitosa* was the predominant coral species along the Mediterranean coast of Israel. Today, it is absent or rare along most of the coast, and common only in a single site in the northern section of the coast (Figure 1).

Oculina patagonica is considered an invading species to the Mediterranean from the Southwest Atlantic. Every summer, the population of this coral in the eastern Mediterranean undergoes a bleaching event (Fine *et al.*, 2001). Most of the colonies survive and recover from bleaching but in recent years there are reports of ~10% mortality. During the bleaching event, colonies of *O. patagonica* cease to grow. Aposymbiotic colonies of *O. patagonica*, inhabiting dark caves, do not experience the bleaching event and maintain growth and calcification but of a very thin encrusting skeleton suggesting that it is not necessarily the lack of symbionts that leads to reduced growth rate, but a general physiological stress that result in bleaching and reduced growth rate.

		<i>Cladocora caespitosa</i>		<i>Oculina patagonica</i>		<i>Polycyathus</i> sp.		<i>Phyllangia mouchezii</i>		<i>Madracis pharencis</i>	
Decade	Location	60's	present	60's	present	'60s	present	60's	present	60's	present
		R. Hanikra	Grey	Grey	White	Grey	White	White	White	Grey	Grey
Akko	Grey	White	White	Grey	Grey	Grey	Grey	White	White	White	White
K. Yam	Grey	White	White	Grey	Grey	White	White	White	White	White	White
Haifa	Grey	White	White	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Atlit	Grey	White	White	Grey	Grey	Grey	Grey	White	White	White	White
Dor	Grey	White	White	Grey	Grey	White	White	Grey	Grey	Grey	Grey
Cesarea	Grey	White	White	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Shfaim	White	White	White	Grey	Grey	White	White	Grey	Grey	Grey	Grey
Palmahim	White	White	White	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Ashdod	White	White	White	White	White	White	White	Grey	Grey	Grey	Grey
Nizzanim	White	White	White	White	White	Grey	Grey	Grey	Grey	Grey	Grey

Figure 1. Distribution and presence of five scleractinian corals along 180 km of the Mediterranean coast of Israel during the 1960s-70s and today. Grey stands for common, white for absence or rare. While cryptic and deep water corals (*Polycyathus* sp., *P. Mouchezii* and *M. pharencis*) show little change, shallow water coral populations changed dramatically. *O. patagonica* established and spread from total absence to very common. *C. caespitosa* reached near extinction within 30 years.

Madracis pharencis is a rather cryptic coral species which can be found symbiotic in niches receiving more light, and aposymbiotic deeper in overhangs and small caves. This species does not experience bleaching event and its population in the Levant basin seem stable in the last three decades. *M. pharencis* is the most dominant coral in the reefs at 25-40 m range.

Ballanophyllia europea is a common species in the Western Mediterranean with some reports of mortality following elevated temperature events (Goffredo *et al.*, 2002). This is a shallow water symbiotic coral, often inhabiting boulders and heavily grazed substrate.

While the response of these corals to elevated temperature is well understood (Rodolfo-Metalpa *et al.*, 2008) their response to ocean acidification has just recently been discussed and only little experimental evidence exist.

It has been demonstrated (Fine and Tchernov, 2007) that skeleton-producing corals (*O. patagonica* and *M. pharencis*) grown in experimental acidified conditions are able to sustain basic life functions, including reproductive ability, in a sea anemone-like form and will resume skeleton-building when reintroduced to normal modern marine conditions (Figure 2). These results support the existence of physiological refugia that allow corals to alternate between non-fossilizing soft body ecophenotypes and fossilizing skeletal forms in response to changes in ocean chemistry. Biomass of the solitary polyps under decreased pH conditions was 1.5 to 3-fold higher than the biomass of polyps in the control colonies. This may be explained by the higher primary productivity (Net and Gross Photosynthesis) that was measured in corals under higher $p\text{CO}_2$. Changes in photosynthesis and calcification as a response to decreased pH were species specific. Gametogenesis in control and experimental corals developed similarly.

Kružić (2007) reported temperature induced polyp expulsion of *Cladocora caespitosa* followed by attachment of skeleton free polyps to the aquarium glass (Kružić, 2007). These observations support the hypothesis that corals can survive as anemone-like polyps and recover when conditions favor calcification.

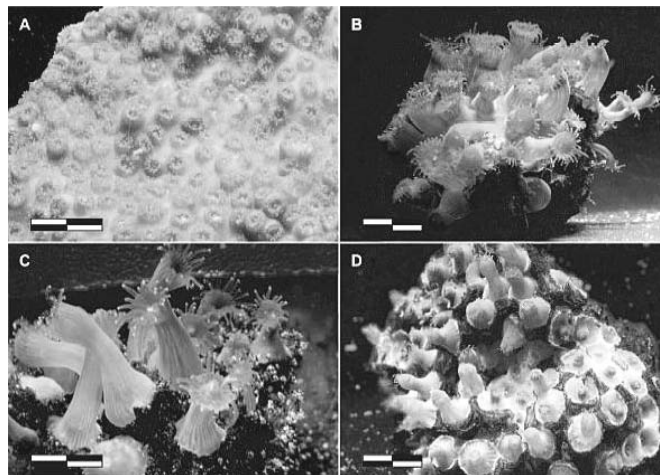


Figure 2. Photographs of (A) Control colony; (B) Slender polyps of corals under low pH conditions; (C) Dissociated colonies with disappearance of the colony connecting tissue, following dissolution of the connecting skeleton; (D) A clone that was transferred back to normal seawater after twelve months as soft bodied polyp.

Hall-Spencer *et al.* (2008) reported total absence of calcifying organisms from a natural CO₂ vent (low pH environment) in Ischia, Italy. Sea grass and algae were very dominant at the high pCO₂ area and corals were restricted to an area out of the influence of the CO₂ vent (Hall-Spencer *et al.*, 2008). Corals (*C. caespitosa* and *B. europea*) that were experimentally transplanted to the vent area survived the vent conditions for over four months (Rodolfo-Metalpa, pers. comm.).

From these observations we conclude that ocean acidification will most likely result in inability of Mediterranean scleractinian corals to calcify and contribute to bioconstruction. While survival of corals under ocean acidification has been proved possible physiologically, it is likely that under predation pressure, the survival of skeleton-less corals is unlikely. Furthermore, ecological forces such as competition with algae and seagrass, which are the big beneficiaries of high pCO₂ environments (Palacios and Zimmerman, 2007), may affect corals at different stages of their life history and demise their settlement success and survival. It should be noted that the examined corals were tested at a relatively low pH, much lower than what is predicted to occur at CO₂ doubling by the end of the century. Therefore, while total dissolution is not projected for the near future, lower growth and lower competitive abilities might take place very soon.

At this point, ocean acidification is not the first in the threats list of Mediterranean coral species; the precedence is reserved to global warming and local anthropogenic stressors. This is especially true for zooxanthellate corals, since shallow waters are more sensitive to temperature rise and coastal pollution. These are thought to cause numerous mass mortality events, coral pathogen outbreaks and viral infections. Some, such as *C. caespitosa* are already threatened in some areas due to invasive species and high water temperatures. Nonetheless, the concomitant shift in community composition as a result of decreased pH as observed in Ischia, Italy (Hall-Spencer *et al.*, 2008) raises concerns to future ecology of the seas in a high-CO₂ world, since it seems that while photosynthetic, non-calcifying organisms are the first to benefit from such change, calcifiers, among them corals, will be the first to suffer. There is no doubt that a combination of ocean acidification and global warming will enhance deteriorating processes of Mediterranean corals.

At this point, global warming and anthropogenic stressors still pose a larger threat to the survival of Mediterranean coral species; some such as *C. caespitosa* are already threatened in some areas. There is no doubt however that a combination of ocean acidification and global warming will enhance deteriorating processes of Mediterranean corals.

OTHER BIO-CONSTRUCTORS UNDER CLIMATIC CHANGE

In the Mediterranean Sea, corals are part of bio-constructing communities that are responsible for some of the most important and diverse ecosystems. Under climatic changes, these ecosystems

might experience deterioration due to reduced calcification rates, which might threaten many other non-calcifying species including many with commercial value. Below are examples of such unique ecosystems that are dependent on bioconstruction.

Coralligenous reefs: characterized by numerous calcareous algae (Corallinaceae and Peyssonneliaceae, red algae) linked together to build a hard structure of up to a few meters in thickness. This ecosystem harbours 650 invertebrate species and at least 30 fish species. It has been shown in a number of studies that calcareous algae are highly sensitive to ocean acidification conditions (Hall-Spencer *et al.*, 2008; Kuffner *et al.*, 2008). Coralline algae were absent from most experimental ocean acidification systems as well as from the Ischia CO₂ vent (Hall-Spencer *et al.*, 2008). Inability of calcareous algae to form bio-constructions might end up in loss of the many species dependent on it. As photosynthetic organisms, these algae may benefit from increased pCO₂ but their role as framework builders is crucial for the well-being of many other organisms including zooxanthellate and cryptic corals. Failure to do so together with natural eroding processes by physico-chemical forces and bio-eroding organisms may shift the present balance from net buildup to net dissolution.

Vermetid reefs: Vermetids are sessile gastropods whose adults have a tubular, irregularly uncoiled shell cemented to hard substrates. In the Mediterranean, two reef-building vermetids – *Vermetus triqueter* and *Dendropoma petraeum* – thrive in intertidal or shallow subtidal zones, forming dense aggregates of colonial individuals with very high densities. Vermetid reefs were described from rocky shores in the southern Mediterranean from Gibraltar Straits and southeastern Spain to the Levantine Basin. Their importance lies in the richness of life they sustain. Their location, at the meso- and infralittoral, places them at risk from coastal development and pollution, with *D. petraeum* recently being recognized as a threatened species in need of protection (Scotti and Chemello, 2000). Ocean acidification may affect both the calcifying abilities of vermetid gastropods and buildup to dissolution balance. Furthermore, the coralline algae involved in consolidating the vermetids buildup are very sensitive to decreased pH as discussed above and this may also reduce buildup rates.

Cladocora banks: *Cladocora caespitosa* is the most important constructional species, which can form structures comparable to tropical reefs (Schumacher and Zibrowius, 1985; Kružić and Požar-Domac, 2003). Some large banks are found in the Ligurian (Morri *et al.*, 1994), Adriatic (Kružić and Požar-Domac, 2003) and Aegean seas (Kühlmann, 1996). Several informal reports suggest a decrease in the population size and spatial distribution of this species in the Mediterranean. Possible causes of this decrease maybe global processes such as global warming and acidification, or more local causes such as competition with invading species (algae and invertebrates).

Effect of elevated $p\text{CO}_2$ on the boron isotopic composition into the Mediterranean scleractinian coral *Cladocora caespitosa*

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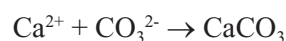
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The Intergovernmental Panel on Climate Change (IPCC) predicts atmospheric CO_2 partial pressure ($p\text{CO}_2$) ranging from 490 to 1,250 ppm in 2100, depending on the socio-economic scenario considered (Prentice *et al.*, 2001). Because one third of anthropogenic CO_2 emissions has been stored in the oceans, ocean pH has already declined by 0.1 unit compared with preindustrial values (Orr *et al.*, 2005) and is predicted to decrease by another 0.4 unit by the end of the century (Caldeira and Wickett, 2003). Seawater acidification will lead to a shift in inorganic carbon equilibria towards higher CO_2 and lower carbonate ion concentrations.

The carbonate ion is one of the building blocks of calcium carbonate (CaCO_3) and changes in its ambient concentration can thus affect the ability of calcifying organisms to precipitate CaCO_3 . Indeed, marine organisms such as coral reefs, foraminifera, coralline algae and mollusks can produce calcareous skeletons or shells following the simplified equation:



At a constant salinity, calcium concentration is rather constant in the ocean, and the calcification process mainly depends on the availability of CO_3^{2-} . The calcium carbonate saturation state follows:

$$\Omega = [\text{CO}_3^{2-}] [\text{Ca}^{2+}] / K^{\text{sp}}$$

where K^{sp} is the stoichiometric solubility product, dependent on temperature, salinity, pressure and on the mineral phase: calcite, aragonite or high-magnesian calcite.

Several experiments have shown a reduction of calcification and size at elevated $p\text{CO}_2$ in tropical corals, mollusks, coralline algae, coccolithophorids and foraminifera (Aegean, 1985; Bijma *et al.*, 1999; Leclercq *et al.*, 2000; Riebesell *et al.*, 2000; Reynaud *et al.*, 2003; Langdon and Atkinson, 2005; Gazeau *et al.*, 2007).

The Mediterranean Sea is an interesting place to study global change and coral calcification because it is land-locked and acts like a miniature ocean, which reacts faster to environmental changes than the open ocean (Bethoux *et al.*, 1990). It is one of the potential hot spots where

critical pH values are reached first and should thus be regarded as a model system in studying changes of seawater chemistry and its effects on marine calcifiers. The study of Mediterranean corals can thus be used to model predictions on how the Mediterranean responds to anthropogenic CO₂ rise and global warming.

In the Mediterranean, some of the gorgonian and scleractinian corals are at their distributional limits with respect to temperature. For example, a mass mortality event of gorgonian and corals has been reported for 1999 at the NW Mediterranean coast and attributed to elevated temperatures (Perez *et al.*, 2000).

C. caespitosa (Figure 1) is endemic and present throughout the whole Mediterranean (Zibrowius, 1980; Peirano *et al.*, 1998). The vertical distributions of these corals range from 5 to 40 m depth.



Figure 1. Colony of *Cladocora caespitosa* (from Roland Graille).

They are also well adapted to turbid coastal environments with high sedimentation rates (Zibrowius, 1974; Peirano *et al.*, 2005). This large distribution, associated with seasonal changes in their environmental parameters, suggests an important acclimation ability of these corals and their associated symbionts to the large panel of light levels (10-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Ligurian Sea, Peirano *et al.*, 1999) and temperatures experienced (from 16 to 31°C along the Israeli coasts, Rosenberg and Ben-Haim, 2002, and from 13 to 26°C in the Ligurian Sea, Peirano *et al.*, 1999).

EXPERIMENTAL DESIGN

An experiment was set up using two independent aquaria with 2 different $p\text{CO}_2$: ambient $p\text{CO}_2$ (ca. 400 μatm), and elevated $p\text{CO}_2$ (ca. 700 μatm). These conditions were maintained during one year (from July 2006). Except $p\text{CO}_2$, which remained constant during the whole year at the two fixed levels, temperature, light and photoperiod were gradually changed in each aquaria according to the measurements made in the bay of Villefranche at ca. 25 m depth (SOMLIT; Service d'Observation en Milieu Littoral, CNRS, INSU). At this depth, seawater temperature varied from 13°C (January-March) to 22°C (July-August) while irradiance varied between 20 and 60 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ in winter and summer, respectively, as measured by Li-Cor 4 π spherical underwater quantum sensor (LI-193SA). In order to maintain a constant seawater temperature, aquaria were installed in a thermostated chamber and, in addition, each aquarium was equipped with one heater connected to electronic controllers (Corema). Two submersible pumps (Micro-jet, Aquarium Systems) ensured water circulation. Light was provided by fluorescent tubes (JBL Solar Ultra Marin Day 39W) at the required irradiance by using plastic mesh.

$p\text{CO}_2$ AQUARIA SET UP AND CARBONATE CHEMISTRY CALCULATIONS

Normal and elevated $p\text{CO}_2$ were obtained by bubbling ambient air and air-rich CO_2 in two batch tanks (110 l). Seawater in the batch tanks was continuously pumped from the Bay of Villefranche at 10 m depth (with a renewal rate of *ca.* 4 l h^{-1}). Each batch tank alimanted one aquarium with a renewal rate of *ca.* 2 l h^{-1} . Partial pressure of CO_2 at the desired level was achieved by using a gas blender (series 850, Signal instrument) in combination with a gas divider (model 821, Signal Instrument). pH was measured every 2 days (*n* total = 170) using an Metrohm 826 pH mobile and an Orion electrode that was calibrated against Sea Water Scale (SWS) buffers: TRIS and AMP (DOE, 1994) at the experimental temperatures. Parameters of the carbonate system ($p\text{CO}_2$, CO_3^{2-} , HCO_3^- , DIC concentrations, and the aragonite saturation state) were calculated from pH (in total scale), TA, temperature and salinity using the Seacarb program.

CORAL COLLECTION

Two colonies of *C. caespitosa* were collected in the Bay of Villefranche (Ligurian Sea, $43^\circ 41' \text{ N}$, $7^\circ 18' \text{ E}$) at *ca.* 25 m depth in July 2006, transported in thermostated tanks to the Laboratory of Villefranche sur mer where the experiment was set up. The colonies were divided into several nubbins. They were carefully cleaned from epiphytes, associated fauna, and sediment, tagged on PVC supports and randomly assigned to one of the two treatments. These nubbins have therefore been maintained during one year under the experimental conditions and have experienced the four seasons. During the whole experiment, no artificial food was supplied to the tanks, but corals were considered naturally fed as the seawater supplied to the aquaria was not filtered.

CORAL STAINING

At the beginning of the experiment, corals skeleton were stained with Alizarin (Figure 2), which was used as a chronological marker. At the end of the experiments, the corals were sacrificed, the tissues were removed (NaOH) and the skeleton deposited during the experiment (i.e. past the skeletal marker) was used for boron isotopic measurements (Figure 2).



Figure 2. Colony of *Cladocora caespitosa* in Alizarin at the beginning of the experiment (left) and at the end of the experiment after tissues removal (right).

BORON

Corals are important archives for geochemical proxies such as O and C isotopes, trace elements, and more recently, boron isotopes. These proxies have increased our understanding of past ocean chemistry and climate change. Understanding environmental effects on biomineralization of corals, and how environmental controls impact isotope and trace element uptake, is essential to get the most information from these proxies.

Because boron isotope uptake in carbonates is controlled primarily by pH, this system may be of value to better understand the mechanisms of coral calcification, and the chemistry of the modified seawater at the site of calcification within the coral, which is difficult to obtain otherwise.

The indication that the boron isotopic composition of carbonates may be controlled by the pH of the parent fluid came out of survey studies of the boron isotopic composition of natural marine carbonates (Vengosh *et al.*, 1991; Hemming and Hanson, 1992). This is due to the fact that the distribution of the two major aqueous species of boron, $B(OH)_3$ and $B(OH)_4^-$ is pH-dependent, and there is a 20‰ isotopic offset between these species (Figure 3).

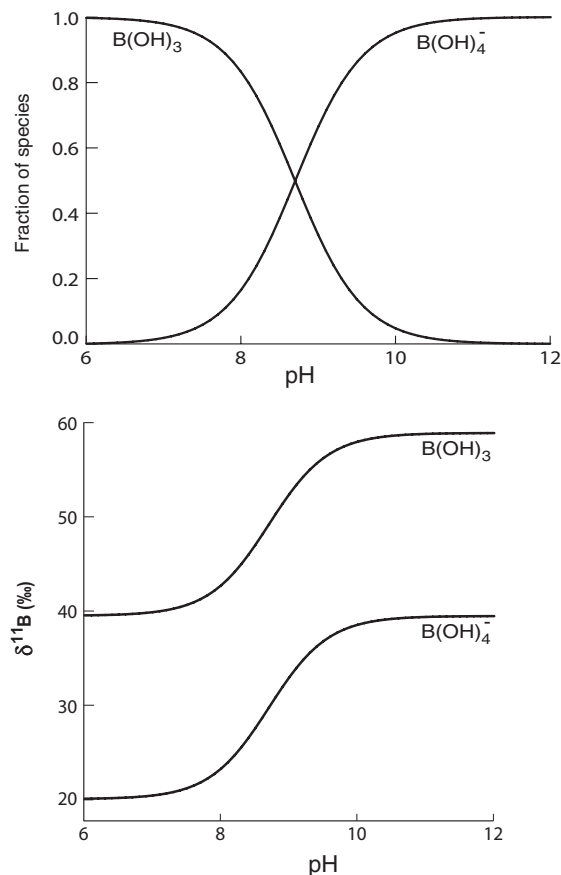


Figure 3. Relative proportion of $B(OH)_3$ and $B(OH)_4^-$ as a function of pH.

There is also strong evidence that only the charged species is incorporated in calcium carbonate. Thus, the boron isotopic composition of the calcium carbonate must change with pH. An increase in ocean pH can be interpreted as a decrease in pCO_2 , so several studies sought to apply the boron isotope paleo-pH proxy to determination of past atmospheric CO_2 . It is noted that paleo-pH reconstruction based on boron isotopes are limited by the residence time of boron in seawater, which is about 13 to 21 million years.

Sanyal *et al.* (1995) utilized boron isotope analysis to investigate climate changes over glacial-interglacial time scales. They found evidence for a higher pH in the glacial surface and deep ocean during the last glacial maximum (LGM), which matched the known atmospheric pCO_2 as recorded in air trapped in ice cores (Petit *et al.*, 1999). This gave credence to the B isotope paleo-pH proxy. Corals should provide an ideal material for recording the paleo-pH of surface water. Their growth rates are high, providing ample sample at high resolution, and they have high concentration of boron (five times higher than in foraminifera). As with any promising new proxy, it is necessary to calibrate it, the goal of this study.

Samples of skeleton for boron isotopic compositions were obtained using a conventional sampling method along a transect (Figure 4). Samples were mounted in epoxy and polished with diamond paste down to 1- μm . They were then ultrasonicated in double distilled water to avoid any contamination.

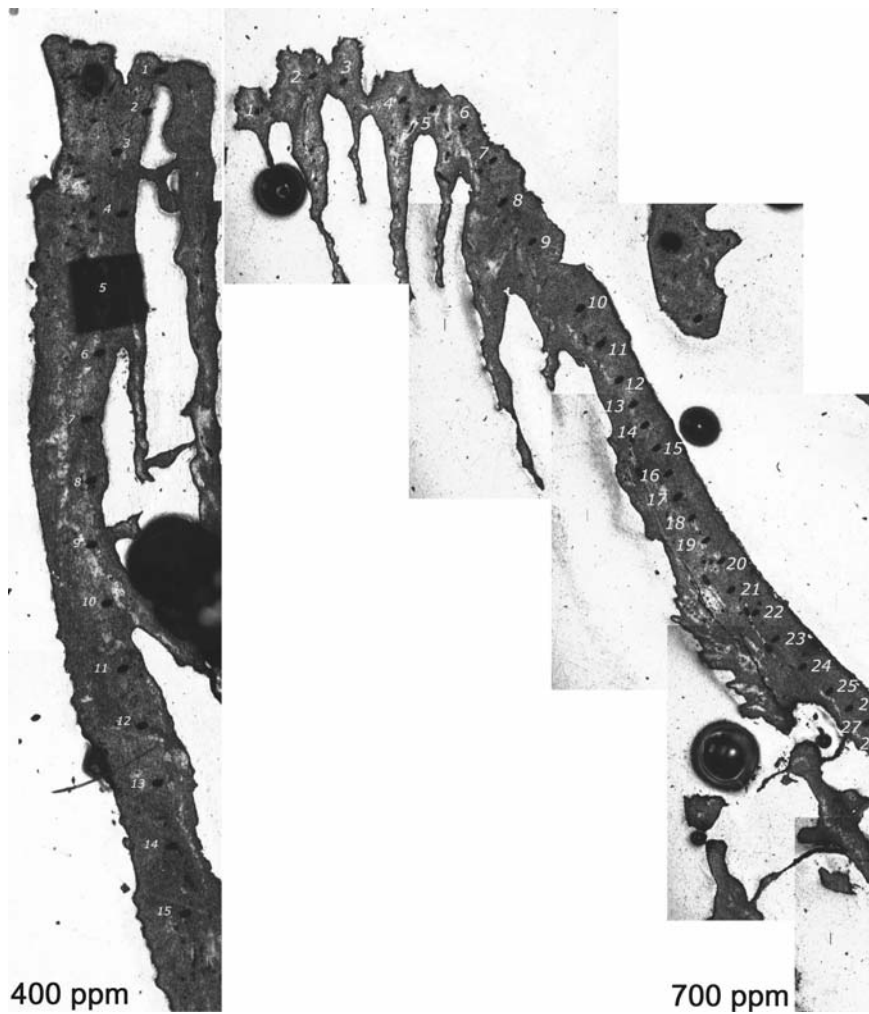


Figure 4. Transect of spots used for $\delta^{11}\text{B}$ measurements.

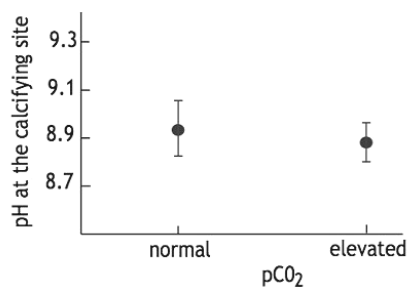


Figure 5. pH deduced at the site of calcification for the two treatments.

RESULTS

Results of $\delta^{11}\text{B}$ measured along a transect on polyps of *Cladocora* did not show any difference in $\delta^{11}\text{B}$ between the treatments (Figure 5): 30.05 ± 1.51 ‰ vs. 29.33 ± 1.28 ‰ respectively for 400 and 700 ppm, corresponding to pH of 8.94 ± 0.11 and 8.89 ± 0.08 .

Despite a difference in the seawater pH (8.1 and 7.9 at normal and elevated $p\text{CO}_2$), the pH calculated from the $\delta^{11}\text{B}$ was the same for the two experimental conditions.

The alkaline pH measured at the site of calcification implies a high control of the animal independently of seawater pH, which raises questions about the use of $\delta^{11}\text{B}$ as a pH-proxy.

Experimental studies of ocean-acidification impacts on Mediterranean seafood species

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ABSTRACT

An experimental facility has been established at the IAEA Radioecology Laboratory to study the effects of ocean acidification (OA) on various marine organisms, particularly from the Mediterranean Sea. Relative to other research groups that are studying OA, our focus is on commercially valuable seafood, beginning with fishes and cephalopods, and not on the 'mega-calcifiers' with shells of calcium carbonate. Thus these seafood taxa are not chosen based on an *a priori* mechanistic hypothesis of thermodynamic control of CaCO₃ precipitation and dissolution. We have used a suite of radiotracers to assess short-term rates of incorporation of essential elements such as Ca and Zn, and trace contaminants that are also expected to increase in the future with industrial growth and increased nuclear power production to mitigate carbon emissions (GESAMP, 2001; IAEA, 2008). Our first results indicate morphological and/or physiological impacts of ocean acidification (OA) among these two commercially important taxa. Thus direct impacts of ocean acidification appear to extend beyond the marine calcifiers.

INTRODUCTION AND RATIONALE FOR OUR STUDIES

In the parlance of risk quadrant analysis the science already undertaken and published by others to assess future impacts of ocean acidification (OA) indicates that we are already in the (*highly significant*: (*very*) *high risk* quadrant for OA and its looming ecological significance. Ocean acidification studies are in their infancy, so clearly more studies are urgently needed, as illustrated by the statement from the Intergovernmental Panel on Climate Change (IPCC) that there is a current gap in knowledge with regard to the effects of OA on marine biodiversity (IPCC, 2007). This gap is starting to be addressed by major international research initiatives, for example the EU Framework 7 Project "European Project on Ocean Acidification (EPOCA)". Meanwhile, it is timely to reflect on the following question:

What scientific findings about impacts of ocean acidification on marine biota are most likely to influence public opinion and the political will to reduce carbon emissions?

Here we can learn from the Stern Review: *The Economics of Climate Change* (2007) and the leverage it has already had on shifting governmental policy with respect to mitigating carbon emissions. The Stern Review has convincingly demonstrated the economic costs of climate change, the costs and benefits of taking action to reduce the emissions of greenhouse gases, and the economic benefits of strong early action on their reductions, ie. within the next 10-20 years. Although it is the extensive and compelling science of climate change that provided the foundation for this economic assessment, it was finally the economic analysis *per se* that demonstrated that timely mitigation is a highly productive economic investment which ultimately persuaded government to begin to move on the climate change issue. The Stern Review also mentions ocean acidification and its “possible adverse consequences on fish stocks”. As ‘profit and loss’ economic arguments ultimately seem to win the day in government decision-making at the highest level, then it is proposed that part of the answer to the question posed above is as follows.

The approach to undertaking ‘policy leverage’ science is a strategic one, rather than being driven solely by mechanistic hypotheses. Namely, it would be useful to experimentally test various taxa that are commercially valued as seafood, in both aquaculture and wild fisheries, for potential negative impacts of ocean acidification on their general viability or growth rates and if found, go on to evaluate economic impacts. For example, such valuations could be used to indicate what a decline this would represent to the aquaculture and fisheries industries in terms of reductions in gross national products. Detriment to these taxa from ocean acidification is easier to evaluate, economically, relative to coccolithophores, the global carbon cycle, or even coral reefs, and the general public can more easily relate to having less fish, oysters or mussels on their table. Such information has greater potential to readily enter broader economic valuation, such as undertaken for the Stern Review, so as to clarify the full social cost of carbon, as propelled by basic economic logic.

RESEARCH PROGRAMME AT THE IAEA MARINE RADIOECOLOGY LABORATORY ON MEDITERRANEAN SEAFOOD SPECIES

With regard to this “needs analysis” our scientific contribution and strategy is as follows. Relative to the focus of other OA research groups, we prefer to place more emphasis on taxa that represent major seafood species (beginning with fishes and cephalopods) but that are not the ‘mega-calcifiers’ with shells or exoskeletons of calcium carbonate. Thus there is no *a priori* mechanistic hypothesis such as thermodynamic saturation-state (Ω) controls for CaCO_3 precipitation and dissolution that underpins the choice of these organisms. Although only exploratory, our investigations are focussed on generating data from taxa that would have greater policy impact, if negative impacts are indicated. Furthermore, our focus is on early life phases (eggs, larvae, and juveniles), which may be the most sensitive stages, as found for metal ecotoxicological studies on fish (Weis and Weis, 1991). Their successful recruitment into fisheries stocks depends on their survival and growth. The experimental parameters we are using are also based on IPCC scenarios for increasing emissions combined with ocean model projections for reductions in surface-water pH for year 2100 (Orr *et al.*, 2005). We reduced the pH of Mediterranean seawater after filtering with activated charcoal, by bubbling CO_2 directly, rather than by adding acid.

We have used a suite of radiotracers to assess short-term rates of incorporation of essential elements such as Ca and Zn, and trace contaminants such as ^{54}Mn , ^{60}Co , ^{65}Zn , Cd, Ag, ^{134}Cs , and ^{241}Am . Some of these contaminants are also expected to increase in the future with industrial growth and increased nuclear power production that may well be installed as one means to help mitigate increasing carbon emissions (GESAMP, 2001; IAEA, 2008). We have chosen to study two fish species, sea bream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*), which currently have the greatest economic value for finfish aquaculture along the Mediterranean and Eastern Atlantic coasts. In 2006, global production of sea bream was 108,000 tons (US \$595 million) and for sea bass it was 60,000 tons (US \$386 million). We are also studying cuttlefish (*Sepia officinalis*), a species of cephalopod. Cephalopod catch was more than 50,000 tonnes in the Mediterranean (FAO, 2000) and is becoming more important as finfish catches decline.

Our first results on the impacts of OA on the eggs and larvae of seabream and cuttlefish are briefly presented in the next section.

Fish: in this experiment, we investigated the consequences of ocean acidification on the embryonic and larval stages of *S. aurata* and *D. labrax*. Fish eggs and larvae were maintained in Mediterranean seawater at normal pH¹ (8.1, $p\text{CO}_2=380$ ppm) and at lowered pH (elevated $p\text{CO}_2$), at a level that could easily occur by the end of this century, 7.9 (650 ppm), and at levels 7.7 (1,100 ppm) and 7.5 (1,800 ppm), which could well occur in the next century. Eggs and larvae were exposed to ^{45}Ca , ^{54}Mn , ^{60}Co , ^{75}Se , $^{110\text{m}}\text{Ag}$, ^{134}Cs , ^{241}Am , ^{65}Zn , and ^{109}Cd dissolved in seawater to assess their uptake kinetics at each of the four pH conditions from the spawning date to the end of the first week of larval development. In addition, 6-month-old juveniles were exposed to ^{45}Ca for one month in order to assess Ca incorporation in calcified body parts such as otoliths, vertebral column, and fins.

Preliminary results indicated that ocean acidification may result in shifts in trace element incorporation in eggs and larvae. Egg uptake of both essential metals (Co and Zn) and non-essential metals (Ag and Cd) was more efficient at normal pH relative to lowered pH, suggesting potential effects on specific fish physiological functions and on retention of toxic elements. In contrast, OA did not alter Ca incorporation in eggs, larvae, and juveniles; calcification mechanisms in this species were thus unaffected by a drop in pH of up to 0.6 pH units.

Cuttlefish: cephalopods play a key role in many marine trophic networks and constitute alternative resources for fisheries. They quickly die after mating leading to population dynamics that are highly dependent on the hatching success of the eggs. Along the European coast, the eggs of *S. officinalis* are characterized by an increasing permeability of the eggshell as they develop, which leads to selective accumulation of both essential and non essential elements in the embryo (e.g. Lacoue-Labarthe *et al.*, 2008a,b). Although temperature and pH are two important factors that affect the metabolism of marine organisms, very few studies have considered the combined effect of both factors on the trace-element bioaccumulation. Here, the effects of pH and temperature were tested through a crossed (3x2) laboratory experiment. Cuttlefish eggs were reared at normal pH (8.10) and at lowered pH values (7.85 and 7.60), and all three treatments were carried out at two temperature levels (16°C and 19°C). Eggs were exposed to ^{45}Ca , ^{54}Mn , ^{60}Co , ^{65}Zn , ^{109}Cd , $^{110\text{m}}\text{Ag}$, ^{134}Cs , and ^{241}Am dissolved in seawater in order to assess their uptake kinetics and distributions among the egg compartments during the full period of embryonic development. It was hypothesized that temperature and pH could affect the embryo metabolic rate and the chemical properties of the eggshell components, which could lead to shifts in a) the accumulation of essential elements (Ca, Co, Mn, Zn) that supply the needs of the embryo and b) the ability of the eggshell to shield the embryo against the entry of non-essential elements (Ag, Cd, Cs, Am).

Our preliminary results indicate that increased $p\text{CO}_2$ (reduced pH) leads to decreased Cd and Zn bioaccumulation in the whole egg, probably due to the effect of pH on the binding properties of the eggshell components. On the contrary, increased temperature increased Zn uptake efficiency, potentially due to a higher metabolic rate in the embryo. Moreover, this work indicates that both increased temperature and reduced pH affect the swelling process and accumulation of Ag and Zn in the hatchlings. These results suggest that the enhanced incorporation of these metals by the embryo, due to reduced pH and higher temperature, may be driven by changes in the osmotic gradient maintained between seawater and the perivitelline fluid and by changes in embryonic metabolism.

WORK NEEDED

Based on current knowledge, the modelling and empirical evaluation of biological and ecological outcomes that are needed include the following:

- i. Scanning for OA effects in a broader range of marine taxa of commercial value in the Mediterranean and also those keystone species that support them, to identify the likely losers and winners in the future;
- ii. collaborating with natural resource economists to begin to evaluate the scale of possible economic losses that would be associated with seafood depletions from ocean acidification,

¹ Note that all pH values are reported on the total scale.

and relaying this advice on potential revenue losses to government and relevant international organisations, e.g., FAO Fisheries, UNEP;

- iii. comparing reduced calcification rates in commercially valuable shellfish in the Mediterranean in order to rank species according to their relative sensitivity to OA, and to advise the aquaculture industry as to the more OA-resilient species.

Impact of ocean acidification on marine shellfish

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ABSTRACT

Ocean acidification resulting from human emissions of carbon dioxide has already lowered and will further lower surface ocean pH. The consequent decrease in calcium carbonate saturation potentially threatens calcareous marine organisms. Among these organisms, shellfish are very important species of a great ecological and also economical value. This paper reviews several reports on the effects of acidification on mollusc species. While numerous studies have shown a significant impact of decreasing seawater pH on shellfish growth and health, most of these studies considered a decrease in pH much higher than the one projected for the end of the century (-0.4 unit). Within this range (8.1-7.7), it appears that acidification does not have a dramatic effect on these species at least over few weeks exposure. However, several studies showed an alteration of metabolic rates and health of these organisms which could be related to shell dissolution and could cause significant loss over longer time-scales. It is therefore of utmost importance to carefully assess the relationship between shellfish calcification/dissolution rates and seawater saturation state with respect to calcium carbonate.

INTRODUCTION

Atmospheric partial pressure of CO₂ (*p*CO₂) will continue to increase beyond the end of this century with predicted values ranging from 500 to 1,000 ppm (parts per million), depending on the considered emission scenario of the Intergovernmental Panel on Climate Change (IPCC, 2007). Because about half of anthropogenic CO₂ emissions has been stored in the oceans since the industrial revolution (Sabine *et al.*, 2004), ocean pH has already declined by 0.1 unit compared with pre-industrial values (Orr *et al.*, 2005) and is estimated to decrease by another 0.4 unit by the end of the century (Caldeira and Wickett, 2003). The dissolution of anthropogenic CO₂ in the ocean shifts the carbonate system equilibrium toward more dissolved CO₂ and less carbonate ions (CO₃²⁻). Besides the direct effect on the buffering capacity of seawater, indirect effects of this anthropogenic CO₂ invasion on oceanic biological processes are expected in the future. Calcifying organisms grow their shells/skeletons from calcium (Ca²⁺) and CO₃²⁻. The calcium carbonate saturation state (Ω) will therefore decrease as

$$\Omega = \frac{[CO_3^{2-}][Ca^{2+}]}{K'_{sp}}$$

, where K'_{sp} is the stoichiometric solubility product, dependent on temperature, salinity, pressure and the mineral phase considered (calcite, aragonite or high-

magnesian calcite). Nevertheless, the surface ocean will remain almost entirely supersaturated ($\Omega > 1$) with respect to calcite and aragonite, except for cold waters that will become undersaturated for aragonite (Orr *et al.*, 2005). Since Broecker and Takahashi (1966) suggested a dependency of calcification rates on CaCO_3 saturation state, several experimental studies have investigated the effect of a $p\text{CO}_2$ increase on the calcification rates of calcareous organisms (Figure 1). Most studies have investigated primary producers and have shown a very large range of responses (calcification decreases by 0 to 92% for a doubling of present $p\text{CO}_2$). Very few studies have focused on the effect of projected $p\text{CO}_2$ levels on the calcification of benthic (Gazeau *et al.*, 2007) and planktonic (Bijma *et al.*, 2002; Bijma *et al.*, 1999; Spero *et al.*, 1997) heterotrophs. Moreover, most studies deal with adults whereas the early life stages of calcifying organisms are generally considered to be more sensitive to environmental disturbances than the adult stages (Royal Society, 2005). In this chapter, I will focus on the important role of shellfish from both ecological and economical perspectives with an emphasis on the Mediterranean and the Black Seas. I will then review the current knowledge on the effect of ocean acidification on these organisms.

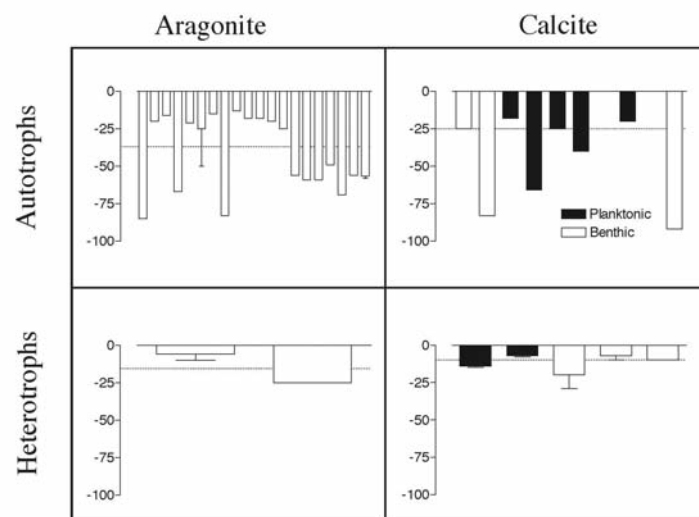


Figure 1. Overview of existing data on the effect of increased atmospheric $p\text{CO}_2$ on calcification rates of marine organisms. Calcifiers were grouped according to their carbon acquisition metabolism (autotrophs vs. heterotrophs) and according to the main mineral phase of their shell or skeleton (aragonite vs. calcite). The Y-axis represents the percent decrease in calcification for an approximate doubling of present atmospheric $p\text{CO}_2$. Planktonic and benthic organisms are represented by black and white bars respectively. Each bar represents a single study ranked chronologically according to the date of publication (updated from Feely *et al.*, 2004; Kleypas *et al.*, 2006). Dotted lines represent the median values.

ECOLOGICAL AND ECONOMICAL IMPORTANCE OF SHELLFISH

Molluscs are ecosystem engineers that govern energy and nutrient flows in coastal ecosystems, provide habitats for many benthic organisms, have strong effect on biodiversity and constitute important resources for birds (Gutiérrez *et al.*, 2003). To the best of my knowledge, no studies have investigated the importance of these species in terms of calcium carbonate production on a global scale. In his seminal paper, Milliman (1993) estimated to 46% the contribution of coastal areas to the global oceanic calcium carbonate production. As most of the data used in his review concerned coral reefs, with almost no data for temperate coastal areas, there is a high probability that this estimate needs to be revised. Very few data are available for temperate shelves, the last extensive study dating from the 1970s in Southern California (Smith, 1972). “Local” studies have since then demonstrated the importance of molluscs in the inorganic carbon cycle. For instance, Chauvaud *et al.* (2003) reported very high rates of calcification by an invasive clam (*Potamocorbula amurensis*) in San Francisco Bay. I have investigated the importance of benthic calcification by molluscs in several ecosystems of the North Sea. Although most of the Dutch North Sea area shows very low rates of calcification ($< 10 \text{ gCaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$), two ecosystems revealed significant calcium carbonate production rates: the Wadden Sea ($\sim 100 \text{ gCaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$)

and the Oosterschelde ($> 1,000 \text{ gCaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$). These two ecosystems are the main sites of shellfish aquaculture in the Netherlands and highlight the increasing role of aquaculture in the global carbonate cycle. With an average annual increase of 7.7% over the last 30 years, global marine shellfish aquaculture production reached 14.1 million tons in 2006 (Figure 2), corresponding to a commercial value of US\$11.9 billion (FAO, 2007). The Pacific oyster (*Crassostrea gigas*) was the most cultivated species in 2006 with a volume of 4.6 million tons or 32.6% of the total shellfish aquaculture production. Around 2,000 mollusc species are found in the Mediterranean and the Black Seas (European Environment Agency <<http://www.eea.europa.eu/>>). Unfortunately, no geographical distribution of species is available yet in the current compilation of the European Register of Marine Species (ERMS <<http://www.marbef.org/data/erms.php>>). Shellfish aquaculture is very important in the Mediterranean and the Black Seas, Italy being the biggest producer far above Greece and France (Figure 3). The major cultivated species are the Mediterranean mussel (*Mytilus galloprovincialis*), the Japanese carpet shell (*Ruditapes philippinarum*) and the Pacific oyster (*Crassostrea gigas*).

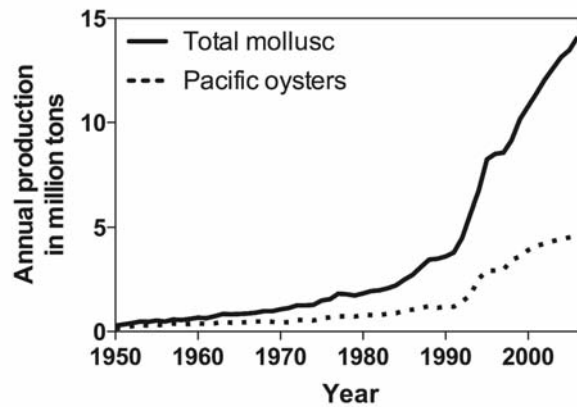


Figure 2. Evolution of mollusc (among which the Pacific oyster (*Crassostrea gigas*)) aquaculture production in the world since the 1950's (data from FAO <<http://www.fao.org/>>).

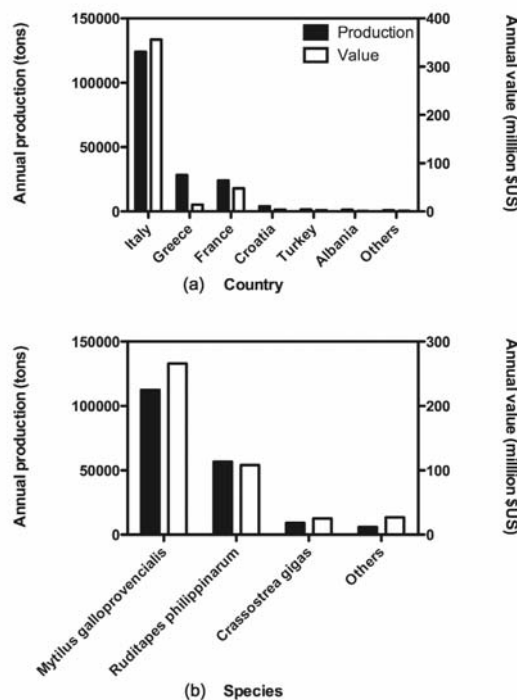


Figure 3. Production (in tons) and commercial value (in million \$US) of shellfish aquaculture in the Mediterranean and the Black Seas, classified by (a) country and by (b) species (data from FAO <<http://www.fao.org/>>).

EARLY STUDIES

Several studies have investigated the effect of low pH on marine molluscs (see evolution of published papers on this topic in Figure 4) although papers previous to 2000 have not dealt with the effect of ocean acidification *per se* and investigated pH decreases far below expected values for the end of the century (-0.4; Caldeira and Wickett, 2003). A non-exhaustive list of such studies is presented in Table 1. All these experiments imply a control treatment with a pH ≥ 8 and perturbed treatments with the highest pH value always far below projected pH values for 2100 ($\ll 7.7$). However, these studies reveal some interesting points. The most sensitive process to low pH appears to be shell dissolution, occurring at pH ≥ 7.5 . As none of these studies report alkalinity data, it is unfortunately not possible to estimate the saturation state of seawater with respect to calcium carbonate although it is very likely that, at this pH level, seawater was undersaturated with respect to aragonite and maybe also with respect to calcite. Nevertheless, no effect on mortality has been observed at pH above 7.5. It seems therefore, based on these results, that pH values expected for the end of the century are not likely to have a dramatic (mortality) effect on mollusc species, at least over exposure times ranging from 8 to 30 days (range of incubation times used in these studies).

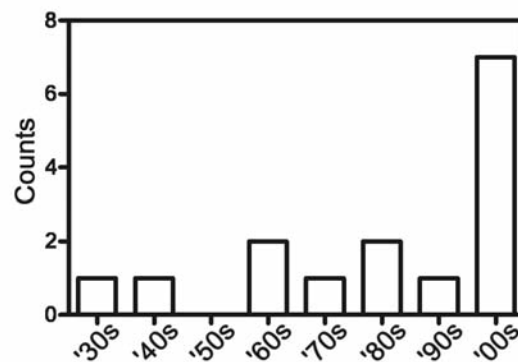


Figure 4. Evolution of the number of studies focusing on the effect of decreasing seawater pH on shellfish since the 1930s.

Table 1. Studies on the effect of low pH before 1990 and the emergence of the acidification issues, adapted from (Bamber, 1990).

Species	Effect	Critical pH	Reference
<i>Ostrea virginica</i>	reduced pumping rate abnormal shell movement	7.0	Loosanoff and Tommers, 1947
<i>Pinctada fucata</i>	increased mortality increased shell dissolution	7.48 7.66	Kawatani and Nishii, 1969
<i>Venerupis decussata</i>	increased shell dissolution inhibition of feeding flesh weight reduction increased mortality behavioural inhibition	7.5 7.0 7.0 6.1-6.4 6.0	Bamber, 1987
<i>Crassostrea gigas</i>	increased mortality	6.0	Bamber, 1990
<i>Mytilus edulis</i>	increased mortality	6.6	
<i>Ostrea edulis</i>	increased mortality	6.9	
All 3 species	growth suppression tissue weight loss reduced shell size shell dissolution abnormal behaviour	7.0 6.5	

THE RISE OF THE OCEAN ACIDIFICATION ISSUE

More recently, in the context of accumulation of anthropogenic CO₂ in the ocean and projected decreased seawater pH, Michaelidis *et al.* (2005) studied the effect of long-term (100 days) moderate hypercapnia on *Mytilus galloprovincialis* (the Mediterranean mussel). However, it has to be stressed that these authors lowered their experimental pH to 7.3, far below expected values for 2100. At a pH of 7.3, they showed a significant reduction in shell growth accompanied by a significant reduction in metabolic rates. These authors explained that long-term hypercapnia has caused a permanent reduction in haemolymph pH and suggested that these organisms increase haemolymph bicarbonate levels derived mainly from enhanced shell dissolution in order to limit the degree of acidosis. Berge *et al.* (2006) studied the effect of increased sea water concentrations of CO₂ on the growth of *Mytilus edulis* over a 45 days period. On one hand, these authors did not find any effect on growth at pH 7.4 and 7.6 in comparison to a control pH of 8.1. On the other hand, a significant decrease of growth has been shown at pH 7.1 and no observable growth at pH 6.7. Moreover, increased mortality rates have been observed at the lowest pH (6.7) while no effect has been observed at higher pH levels. Bibby *et al.* (2007) examined the effect of an important decrease in seawater pH (from 7.97 to 6.45; N. B. S. scale) on induced morphological – defences in the form of shell thickness – of the intertidal gastropod *Littorina littorea*. In the presence of predators, the organisms were unable to increase their shell thickness at pH 6.45. Interestingly, this study also revealed a capacity of this species to compensate for this decrease in morphological defence capacity by an increased behavioural response as shown by the increased capacity of these organisms to avoid predators at low pH. Gazeau *et al.* (2007) investigated, for the first time, the effect of increasing pCO₂ on calcification rates of two ecologically and commercially important species: the edible mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*). These authors showed a linear decrease of calcification rates, as estimated by the alkalinity anomaly method¹, for both species (Figure 5). Based on these linear relationships, they estimated that calcification rates of mussels could decrease by 25% by the end of the century following the IPCC IS92a scenario (~740 ppmv in 2100) while calcification rates of oysters could decrease by a lower value (-10% in 2100). Based on Gazeau *et al.* (2007), it is very likely that mussel calcification is directly dependent on the saturation state of seawater with respect to aragonite as shown on Figure 6, with null net calcification rates (gross calcification – dissolution) at $\Omega_{\text{aragonite}} = 1$. These results contrast with the ones of Berge *et al.* (2006) who showed no effect of decreasing pH down to 7.4 on mussel (*Mytilus edulis*) growth as measured by length increase. The apparently contradictory results between these two studies can be explained in a number of non-exclusive ways. First, it is obvious that the incubation times used in Gazeau *et al.* (2007) did not allow any potential adaptation by the organisms in comparison to the 45 days incubation period used by Berge *et al.* (2006). As mentioned previously, Bamber (1990) and Michaelidis *et al.* (2005) reported a decline of metabolic activity (e.g. respiration rates) under hypercapnic conditions over longer incubation periods (> 1 month), which was not found in Gazeau *et al.* (2007). A decrease in respiration implies a decrease of internal pCO₂ and therefore an increase of the capacity of the organism to fix CaCO₃. Second, the methods used in the two studies differ significantly. Berge *et al.* (2006) only measured shell length increments, while a reduction of shell thickness, and consequent decrease of shell weight, is very likely (Bamber, 1990). Finally, Bibby *et al.* (2008) showed an effect of acidification (from control pH of 7.8 to pH levels of 7.5 to 6.7) on the physiological condition of *Mytilus edulis*, attributed to an increased level of Ca²⁺ in the haemolymph due to shell dissolution. This theory is also used by Beesley *et al.* (2008) to explain a significant decrease in health as measured by the Neutral Red Retention assay while no impact has been shown on tissue structure. It has to be stressed that in these two studies, no calcification/dissolution measurements have been done precluding the validation of this theory.

¹ The alkalinity anomaly method is based on the fact that the precipitation of 1 mole of CaCO₃ implies the consumption of 2 moles of HCO₃⁻, therefore decreasing alkalinity by 2 equivalents. Calcification rates were therefore estimated based on the difference between alkalinity values at the start and at the end of the incubation period (2 hours), assuming that no other processes have an impact on alkalinity.

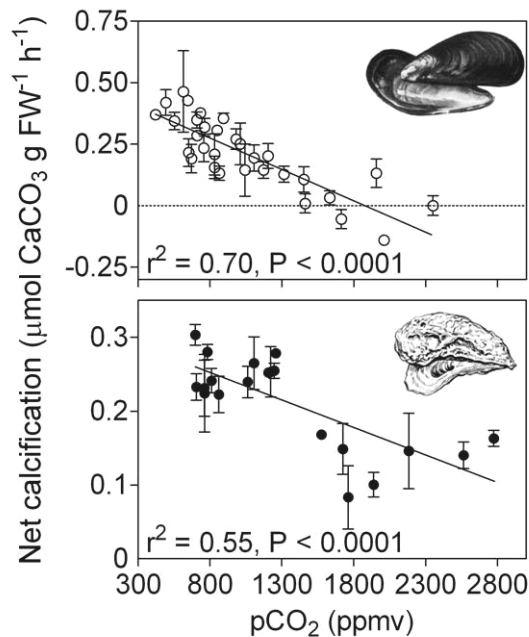


Figure 5. Net calcification rates of *Crassostrea gigas* (Pacific oyster) and *Mytilus edulis* (blue mussel) as a function of seawater partial pressure of CO₂ (adapted from Gazeau *et al.*, 2007).

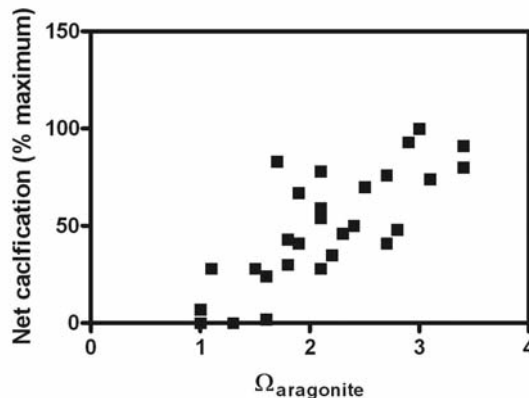


Figure 6. Net calcification rates (percentage of maximal rate) of *Mytilus edulis* (blue mussel) as a function of the seawater saturation state with respect to aragonite ($\Omega_{\text{aragonite}}$).

From an ecological point of view, effects on early life are of pivotal importance as 1) they are generally more sensitive to environmental disturbances, 2) most benthic calcareous organisms possess planktonic larval stages and 3) fluctuations in these larval stages due to high mortality rates regulate population size (Kurihara *et al.*, 2007). Unfortunately, only two studies focused so far on the effect of ocean acidification on an early developmental stage of mollusc species: Kurihara *et al.* (2007) and ours. Kurihara *et al.* (2007) showed that a pH decrease to 7.4 caused a significant decrease of *Crassostrea gigas* larval development success. Indeed these authors showed a deleterious impact of such a pH decrease on shell mineralization, as only 30% of the larvae exposed to high CO₂ were fully mineralized as compared to 72% in the control treatment. It has to be noted that at this pH, the seawater was clearly undersaturated with respect to aragonite (0.68). Gazeau *et al.* performed a study on the development of *Mytilus edulis* larvae. They present experimental data showing that the growth of planktonic mussel (*Mytilus edulis*) larvae is affected by a decrease of pH, especially when the water becomes undersaturated with respect to aragonite, the main calcium carbonate mineralogical form of its shell. At pH ~7.8, in the range of values expected for the end

of the century, seawater was still oversaturated with respect to aragonite and although there were no significant effects on both hatching and mortality rates during the first two days (eggs to D-shape larvae) or during the following period prior to settlement (2 to 14 days), shell growth decreased by 15.6 and 10% during these two growth periods respectively. Moreover, a pH of ~7.6 and the associated slight undersaturation with respect to aragonite, had very important effects on both hatching and growth rates, during the first two days when the shell is actually produced. Hatching rates decreased by 24% while growth in terms of length and weight decreased by 38% and 73%, respectively.

CONCLUSION

Based on these different studies, several conclusions emerge:

- A decrease of seawater pH by 0.4 unit as expected for 2100 does not seem to have an effect on shellfish mortality rates over several weeks exposure.
- Shell dissolution seems to be a critical process, which is suspected to impact mollusc physiology, immune system (Bibby *et al.*, 2008), metabolic rates (Michaelidis *et al.*, 2005) and health (Beesley *et al.*, 2008).
- Study of Gazeau *et al.* (2007) suggests that shell net dissolution occurs as soon as the water becomes undersaturated with respect to the main mineralogical form produced by the organism. More data are needed to carefully assess the effect of decreased pH following a realistic scenario (a maximal decrease of 0.4 unit for the next 100 years) on this process and the potentially related physiological deteriorations on marine shellfish species.
- Only one study focused so far on the effect of ocean acidification on a typical Mediterranean species (*Mytilus galloprovincialis*; Michaelidis *et al.*, 2005). More data are needed in this area.
- Very few studies focused on the planktonic larval stages of marine shellfish although significant effects have been demonstrated for two different species.
- Seawater temperature increase will be an additional stressor for marine organisms in the next decades, the combined effect of these two perturbations needs to be assessed.

Acknowledgements

Studies from Gazeau *et al.* presented in this review have received support from the Netherlands Organization of Scientific Research and contribute to the European Project on Ocean Acidification (EPOCA).

Ocean acidification and its impact on the early life-history stages of marine animals

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ABSTRACT

The world's oceans are slowly becoming more acidic and profound changes in marine ecosystems are certain. Expected changes for the coming century will have significant effects on marine animals, especially those lacking adequate physiological buffering capacity and/or with calcareous skeletons such as echinoderms. In addition, alarmingly little is known about the long term impact of predicted pH changes on marine invertebrate larval development. Data currently available shows that the impact of ocean acidification (OA) on marine animals is not easy to predict (e.g. calcareous *v.s.* non-calcareous species, negative *v.s.* positive effects), furthermore it is species-specific even within closely related taxa and operates in synergy with other environmental parameters (e.g. temperature, food availability). An holistic approach on several generations including all phases of individual life cycles is required and essential. Further studies should focus on an established and robust frame of reference for life-history analyses, including temporal as well as developmental stages. Moreover, it is important to work in realistic abiotic (e.g. pH, temperature) and biotic (e.g. nutrient/food concentration) conditions, for example within a range of seawater pH predicted to occur by the year 2100 (Δ pH \approx - 0.2 to - 0.4 units) regulated by manipulation of environmental CO₂ levels. Our view is that we need studies that embrace a wide range of carefully selected taxa and that also include careful consideration of life-history strategies exhibited by marine organisms. These considerations are illustrated by examples from our own research.

BACKGROUND

Many years ago Knutzen (1981) stressed the potential dangers of OA and the need for studies involving all life-history stages, longer exposure times, and especially the need to focus on calcareous organisms (calcifiers). However, until recently this call went largely unheeded and even today there are relatively few published reports on this topic (Harley *et al.*, 2006). It is surprising then that the impacts of OA on the delicate embryonic and larval stages that are essential for recruitment and population maintenance have largely been ignored. Until now, only the work of Kurihara and colleagues (Kurihara and Shirayama, 2004; Kurihara *et al.*, 2004; 2007; Kurihara and Ishimatso, 2008) has focused on these early life-history stages in animals. These authors showed significant deleterious effects of CO₂-induced acidification on early larval development in echinoderms, molluscs and crustaceans, albeit at pH levels predicted for the year 2300 (i.e. Δ pH -0.7 units) and short term exposure.

OA IMPACT IS NOT EASY TO PREDICT AND IS SPECIES-SPECIFIC

Marine animals represent an enormous range in size, structural diversity and in adaptations to different modes of existence. For example, amongst marine calcifiers, calcification mechanisms differ considerably. It is frequently overlooked that echinoderms are the only invertebrates (thus like chordates in this respect) with endoskeletal calcified support: their skeletons are enclosed within at least one epithelial integument (Wilt, 1999). Other typical marine invertebrate calcifiers (molluscs, crustaceans, worms) produce their skeletons as epidermal secretions with direct contact to the environment. Furthermore, amongst echinoderms, larval morphology is different between classes. In sea urchins and brittlestars the larvae have an extensive endoskeleton while in sea star larvae there is no skeleton. However, all adults have a distinctive endoskeleton that begins its construction within the larval body at the later stages of larval life. This means that there are two quite distinct stages of skeletogenesis, the larval phase, present in some echinoderms, and the adult phase, present in all echinoderms. It is not unreasonable then to expect that impact of OA will differ between taxa.

Table 1 summarizes our data on impact of OA on a range of marine animals. Low pH affects larval survival, developmental dynamics and adult fecundity. It is important to note that these effects are species-specific, even in closely related species, and that the effects observed are not all negative. For example, a dramatic effect on survival and development is observed in the brittlestar *Ophiothrix fragilis* (Dupont *et al.*, 2008) when more subtle effects are observed in other brittlestars (see Example 1 below). In echinoids, a negative effect on survival is observed in some species (e.g. *Brisopsis*) while other (*Strongylocentrotus*) seem unaffected by OA. It is also important to remark that the tunicates we have studied show a positive response to OA with a higher survival rate, faster development and higher fecundity.

Table 1. Summary of the effect of OA on larval development.

Species	Survival	Developmental dynamics	Fecundity
CRUSTACEANS			
<i>Acartia tonsa</i>	=	-	.
ECHINODERMS - OPHIUROIDS			
<i>Amphiura filiformis</i>	--	-	.
<i>Ophiocomina nigra</i>	--	-	.
<i>Ophiothrix fragilis</i>	0	-	.
<i>Ophiura albida</i>	--	-	.
ECHINODERMS - ASTEROIDS			
<i>Asterias rubens</i>	=	-	.
ECHINODERMS – ECHINOIDS			
<i>Brisopsis lyrifera</i>	--	-	.
<i>Echinus esculentus</i>	--	-	.
<i>Paracentrotus lividus</i>	+	-	.
<i>Strongylocentrotus purpuratus</i>	+	-	.
<i>Strongylocentrotus droebachiensis</i>	+	-	.
TUNICATES			
<i>Asciidiella aspersa</i>	+	+	.
<i>Ciona intestinalis</i>	+	+	.
<i>Oikopleura dioica</i>	+	+	+

Consequences of OA (pH 7.9 & 7.7) on survival, developmental dynamics and fecundity in a range of marine larvae (= no effect ; - negative effect ; + positive effect ; . not tested).

Example 1 - Impact of OA on survival in two brittlestars.

In *Ophiothrix fragilis*, OA induces dramatic mortality after one week post-fertilization (Figure 1B) due to larval and skeletal malformations (Figure 2; Dupont *et al.*, 2008) while more subtle effects are observed in *Amphiura filiformis* in which some larvae (although 20% fewer than in the control) survive to the juvenile stage.

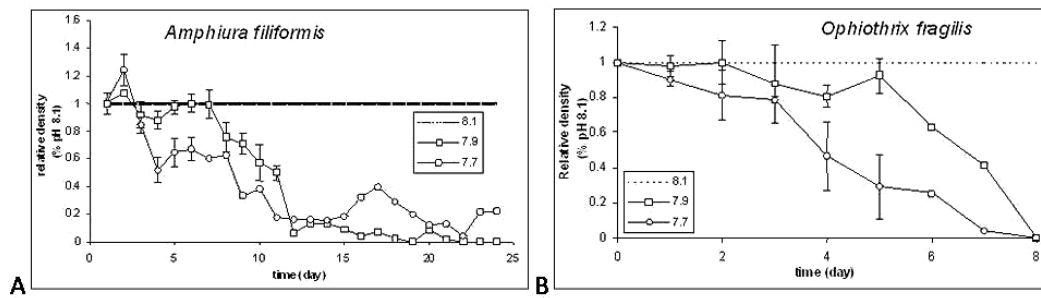


Figure 1. Impact of OA on larval survival in two brittlestar species. Larval relative density (calculated as the percentage of the control 8.1) through time post fertilization in two species of brittlestars (A) *Amphiura filiformis*; B) *Ophiothrix fragilis*) exposed to OA (pH 7.9 and 7.7).

These data highlight the danger of extrapolation from single species, even within closely related taxa. Thus we suggest that a wide range of species should be investigated with different life-history traits, habitats (both adult and larval), physiology, morphology, adaptation potential, etc., without being too prescriptive.

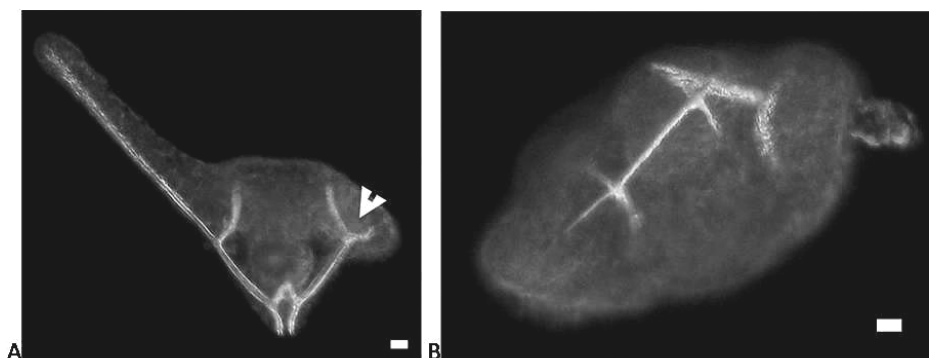


Figure 2. Impact of OA on the brittlestar *Ophiothrix fragilis*. Example of abnormal larvae of *Ophiothrix fragilis* raised at low pH using polarized light to highlight skeletal malformations. A) Asymmetric larvae (2 days pluteus at pH 7.7) with a shorter posterolateral rod (arrowhead); B) Abnormal larvae (2 days pluteus at pH 7.7). Scale bar = 10 μ m.

OA IS NOT ONLY A CALCIFICATION ISSUE

Very little is known regarding processes other than calcification. This is unfortunate because it is clear that a variety of biological functions will be affected by OA. For example, calcium and other ion-transport based phenomena are vital for many physiological processes (i.e. ciliary activity, muscle contraction, neural signaling and integration) and the onset of development in most species studied is triggered by one of the largest and longest calcium transients known (Whittaker, 2006; 2008). Moreover, some calcium channels have been shown to be very sensitive to pH (Mignen and Shuttleworth, 2000). Thus in addition to skeletogenesis, it might be predicted that fertilization and early development events will be sensitive to OA.

Within the pH range of changes expected for the coming century, skeletogenesis is affected by OA in some species (abnormalities, asymmetry, etc., see Example 1), while other calcifying larvae apparently show few problems with skeletogenesis (see Examples 3 & 4 below). In several sea urchin larvae, the skeleton develops normally with only a delay in developmental dynamics (see Examples 2 & 4 below). On the other hand, other parameters seem to be affected by OA such as feeding efficiency (see Example 5 below).

This leads us to the conclusion that it is vital to address wider implications than skeletogenesis. While it is likely that marine calcifiers will be affected, it is essential not to overlook the fact that pH changes will impact a whole raft of other vital physiological processes.

OA IMPACTS ALL ASPECTS OF THE LIFE-CYCLE

At the species level, OA appears to have a direct impact on fecundity, fertilization success, recruitment and individual performance at all stages of the life cycle (Figure 3). For example, in sea urchins, it was shown that OA may affect fertilization success (Havenhand *et al.*, 2008), larval development (Kurihara and Shirayama, 2004; Kurihara *et al.*, 2004; 2007) and adults (Shirayama and Thornton, 2005; Miles *et al.*, 2007). OA may also have indirect impacts. For example, long term exposure of adults to OA may affect future larval performance (see Example 2 below). It is then essential not only to study the direct impacts of OA but also transfer processes at all phases of the life-cycle.

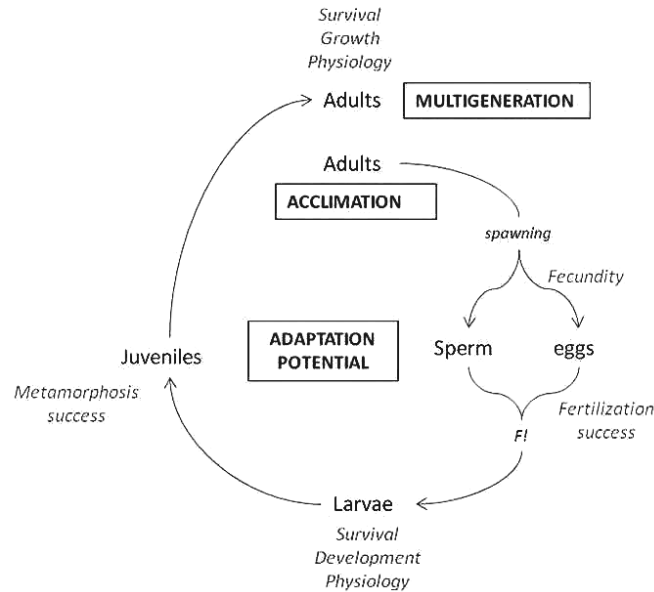


Figure 3. Impact of OA on species life-cycle.

Example 2 – Impact of adult exposure to OA on larval performance in the sea star *Asterias rubens*.

Direct exposure of fertilized eggs of the sea star *Asterias rubens* to OA (pH 7.9 – 7.7) does not affect survival or recruitment but does induce a delay in developmental dynamics (Table 1). Exposure of the adults to OA (pH 7.7) for the four months prior to the reproductive period has indirect consequences on future larval development. Larvae cultured at low pH (7.7) experienced high mortality, abnormal development (Figure 4) and were unable to feed.

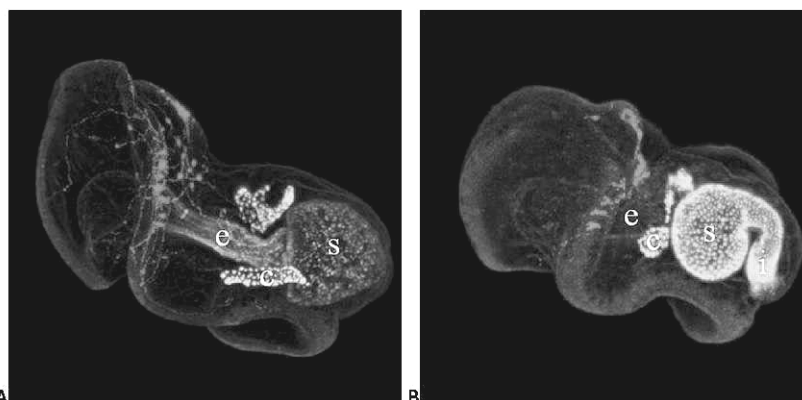


Figure 4. Impact of adult exposure to OA on larval development in the sea star *Asterias rubens*. Confocal images of bipinnaria larvae of *Asterias rubens* (day 7) after pre-exposure of the parents for 4 months prior to spawning. The Insulin Growth Factor II (IGFII) is revealed using antibody labeling. In the control (A), IGFII is expressed in the esophagus (e) and the coelom (c) while at low pH (7.7, B) it is expressed in the stomach (s), the intestine (i) and the coelom (c). This later expression is associated with a lack of feeding ability.

OA WORKS IN SYNERGY WITH OTHER ENVIRONMENTAL STRESSORS

Little is known about the synergistic effects of OA with other stressors. For example, global warming has affected marine animal distribution with the enhanced risk of local extinction of species and even ecosystems. Future scenarios indicate a threat to marine life through the specific or synergetic effects of both OA and temperature. Our data show that some effects of OA are only revealed when combined with an increase in temperature (see Example 3 below). To understand the full impact of OA, it is then vital to combine stressors in further studies.

Example 3 – Impact of OA and temperature on the development of the sea urchin *Strongylocentrotus purpuratus*.

When cultured at 14°, larvae of *S. purpuratus* show little sensitivity to OA in the studied range of pH (8.1-7.7). However when raised at 16° in the same pH ranges, development of larvae is delayed (Figure 5) and they failed to reach the juvenile stage.

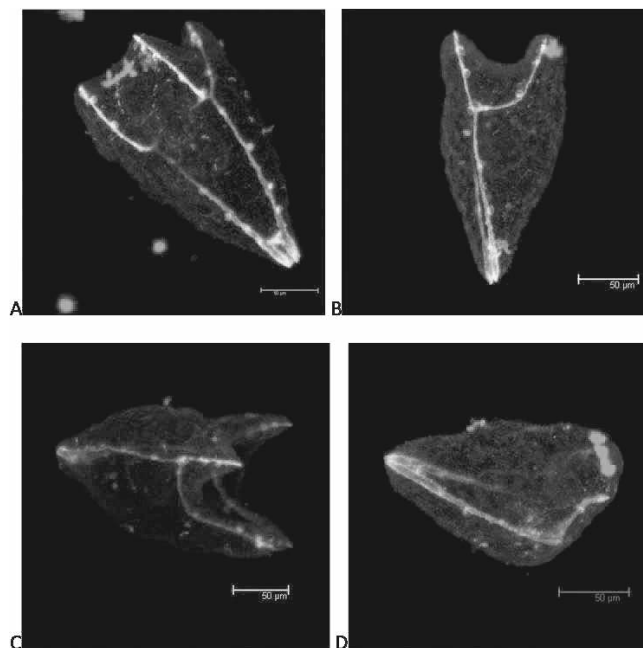


Figure 5. Synergetic effect of OA and temperature on *Strongylocentrotus purpuratus* larvae. Confocal images of the skeleton (Primary Mesenchyme Cells – PMC) revealed by PMC-specific cell surface marker, MSP130, Anstrom *et al.*, 1987) in 3 day pluteus of *Strongylocentrotus purpuratus* larvae raised at different temperatures and pH: A) 14°C/8.1; B) 14°C/7.7; C) 16°C/8.1; D) 16°C/7.7. If the skeleton is normal in all conditions, its development is delayed at low pH and high temperature.

TEMPORAL DYNAMICS V.S. DEVELOPMENTAL STAGES

Supposedly deleterious effects after short exposure to OA are perhaps not relevant in terms of individual fitness and may not reflect the true impact of the contribution to the next generation.

It is essential to assess potential effects over the complete period of development using morphological and functional physiological assays as key issues/metrics. For example in echinoderms, adult rudiment development, onset of feeding, respiration, etc., are critical measures for the impact of OA on life-history processes.

OA will impact dynamics of many biological and developmental processes, (see Example 4 below). To assess the real impact of OA on individual fitness, it is essential then to take into account the whole period of development from egg to juvenile, and so assess affects on *stages* of development rather than only *time* of development and avoid conclusions based on punctual observations (Troedsson *et al.*, 2007). For example, if the treatment induces a delay in development, it takes more time to reach each developmental stages (I to V on the Figure 6). A measurement at time t will lead to the conclusion that the treatment has a strong negative impact on the observed parameter (e.g. Size, skeletogenesis, etc.) when this observation is simply a consequence of a delay in development

(larvae are at stage IV in the control but only at stage III in the treatment) with no relevance to individual fitness/success. Although if settlement is delayed in consequence AND the larva spends more time in the water column then there may be indirect impacts on fitness (see also comments below).

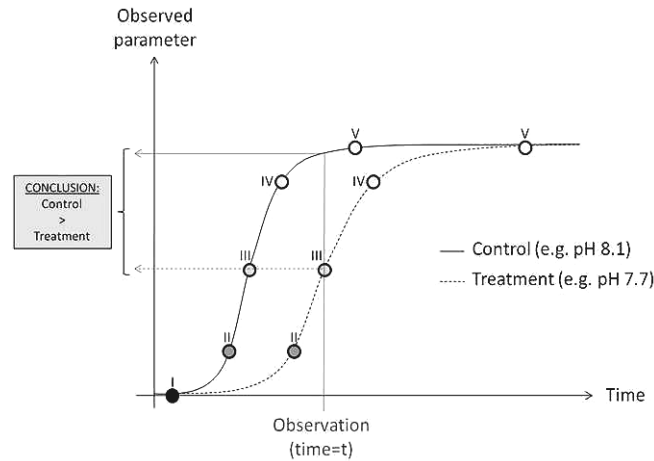


Figure 6. Relation between time and stage of development.

Example 4 – Impact of OA on long term development of *Strongylocentrotus droebachiensis*.

Fertilized eggs of the sea urchin *S. droebachiensis* were cultured at three different pH values (control 8.1, 7.9 and 7.7). After 21 days, larvae raised at low pH were significantly smaller than those raised at normal pH (Figure 7). At normal pH (8.1) a well developed rudiment is present while it is less developed at pH 7.9 and entirely absent at pH 7.7. This effect is often interpreted as a negative impact of OA on larval growth, development and skeletogenesis (i.e. see Kurihara and Shuriyama, 2004; Kurihara *et al.*, 2004). Thirty days later, juveniles were present in all cultures (Figure 7). If smaller at low pH, the proportion of successful metamorphoses is significantly higher at low pH (6 to 14 times more juveniles). This example illustrates the fact that punctual observations can lead to misinterpretation of the real impact of OA. What could be interpreted as a negative impact at day 21 (smaller larvae) may be considered later as a globally positive impact (higher number of successful larvae at day 50). It is then essential to study the entire life cycle to assess real impact of OA in terms of a trade-off between individual (i.e. recruitment success, developmental speed, etc.) and ecosystem (i.e. predation rate, settling success, etc.). For example, at low pH, a higher proportion of larvae successfully reach metamorphosis but since it takes more time to reach this stage, these larvae may be disadvantaged. Planktonic mortality is high and increasing the time a larva spends in the plankton increases the chance of loss by predation and/or by delay in the opportunity to settle in a high quality habitat (Miner, 2005; Elkin and Marshall, 2007).

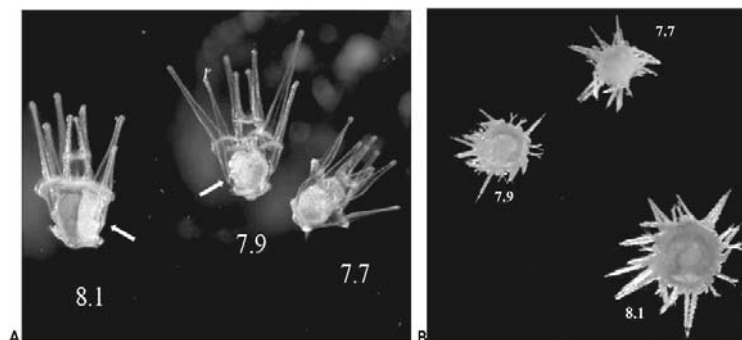


Figure 7. Impact of OA on development of *Strongylocentrotus droebachiensis*. *Strongylocentrotus droebachiensis* larvae cultured at different pH (control 8.1, 7.9 and 7.7). A) Late pluteus larvae at day 21 (rudiment = white arrow). B) Juveniles at day 50.

ADAPTABILITY POTENTIAL TO OA

Lastly, nothing is known about intraspecific variability, and hence the capacity to adapt, in response to OA. This is critical for understanding the long-term resilience of marine communities to acidification. Genetic variability determines how physiological mechanisms respond during acclimation (within individual) and adaptation (between generations). It is essential to assess individual variability.

For some parameters (e.g. feeding rate, respiration, enzymatic assays, RT-PCR), practical limitations imply that at present it is only possible to work on large samples (10 to several thousand larvae per experiment). It is then difficult to interpret the results in terms of selection/adaptation potentiality. This makes it impossible to assess the difference between a treatment inducing a moderate decrease for the measured parameters in a vast majority of the larvae (Figure 8 treatment A) from a treatment inducing a strong decrease in half of the larvae (Figure 8 treatment B). These two types of consequence are really different in terms of adaptation/selection potential. A type A effect has a low potential for adaptation (only few larvae are "normal" in this condition) while in type B, 40% of the larvae can pursue normal development. The long term consequences may be completely different in a way that the measured parameter is unable to predict. It is therefore essential to work at the individual level to assess intraspecific diversity.

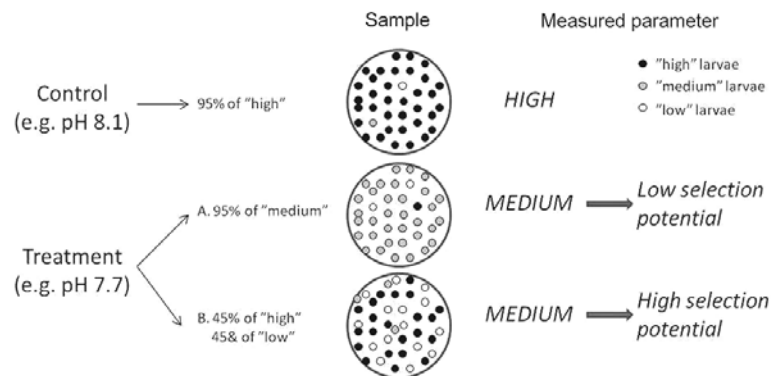


Figure 8. Assessment of adaptability potential to OA.

Example 5 – Impact of OA on feeding efficiency in the sea urchin.

Using both individual and population assays (Figure 9), *S. droebachiensis* larval feeding efficiency at low pH is significantly lower than controls. Nevertheless, only individual assays reveal intraspecific variability. At low pH, larvae present high variability in the number of esophageal contractions, a proxy of feeding efficiency, some individuals being comparable to the control. The lowest feeding rate can then be interpreted as a mixture of "good" (able to adapt to low pH condition) and "bad" larvae (unable to adapt to low pH). In consequence, *S. droebachiensis* possess a high selection potential to OA.

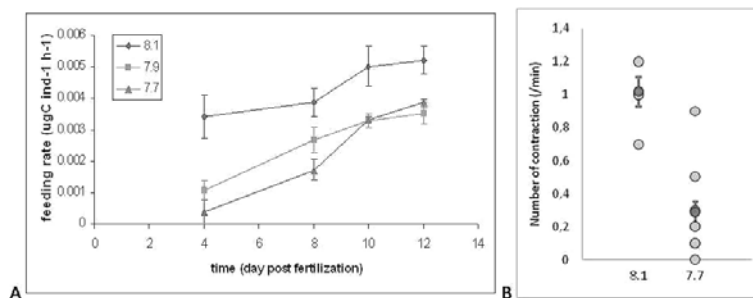


Figure 9. Impact of OA on feeding rate in *Strongylocentrotus droebachiensis* larvae. Measurement of feeding efficiency in *S. droebachiensis* larvae using two different methods. A) Feeding rate of larvae raised at different pH (control 8.1, 7.9 and 7.7) between days 4 and 12 and estimated by the quantity of food eaten by 15 larvae over a period of 24 hours. B) Feeding efficiency of larvae raised at different pH (control 8.1, 7.9 and 7.7) at day 12 and estimated by the number of contraction of the esophagus per minute over a 10 minutes individual assay.

The ultimate measurement of adaptability potential to OA is to observe microevolution at low pH. It is important then to perform multigenerational studies to quantify adaptability and microevolution potential. Studies on species with a short life cycle are then more tractable.

Example 6 – Impact of OA on the development of the copepod *Acartia tonsa* over several generations.

The main impact of OA (pH 7.7) on the development of the copepod *Acartia tonsa* is an increase in the generation time (Figure 10). However, at the second generation at low pH, the generation time returns to a value similar to that of the control, probably a consequence of artificial selection of animals adapted to low pH.

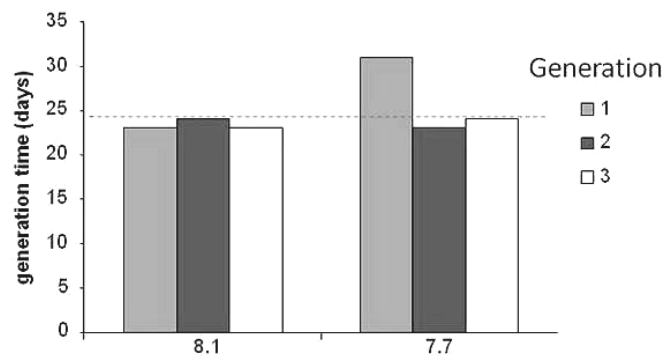


Figure 10. Impact of OA on the generation time of the copepod *Acartia tonsa*. Generation time of the copepod *Acartia tonsa* raised at two different pH (control 8.1 and 7.7) over three generations.

CONCLUSIONS

Dramatic environmental changes in the past (e.g. the Permian extinction, see Knoll *et al.*, 2007) were not only responsible for wiping out large numbers of taxa but also creating opportunities for those with more flexible and robust regulatory systems. We need to take into account parameters such as generation time, adaptation potential, as well as life-history strategies into our predictions of future changes.

Our present knowledge on the impacts of OA on early life-history stages in marine animals is largely based on experimental work with species maintained in short-term cultures, often exposed to abrupt and extreme changes in pH. Little is known of adaptability potential, synergetic effects with other stressors (e.g. temperature), and how the response to OA is transferred from the organism(s) to the ecosystem level or the replacement of OA-sensitive to OA-tolerant species.

More long term experimental data for likely pH values in the coming century (pH>7.6) are needed in order to make meaningful predictions of broader ecosystem consequences and define critical thresholds for tolerable pH decline.

The integration of molecular and biochemical mechanisms into whole organism functional networks and their capacity is thus a crucial element in understanding cause and effect at the ecosystem level. This requires knowledge of the molecular, cellular and physiological mechanisms of OA effects and their whole organism consequences (Pörtner, 2008).

Last but not least, if we want to predict future consequences of OA on marine ecosystems, it is important to think and to work at level higher than the organism (e.g. mesocosm experiments). We have already stressed the danger of misinterpretation of results. For example, a higher success of metamorphosis at low pH may be detrimental for the species if associated with an increase of developmental time (and then larval mortality in natural conditions due to higher predation). To have a realistic picture of future consequences of OA, we need to take into account processes such as competition, predation, parasitism, trophic relationship, etc.

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Impact of acidification on pelagic calcifying organisms in the Mediterranean Sea

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The carbonate system of the Mediterranean Sea and the response of the highly adapted organisms to the rapid CO₂ increase are both poorly understood. Coccolithophores (planktonic photoautotrophic microalgae) are the dominant calcifying organisms in the Mediterranean today. Their calcite plates (coccoliths) present a large variability in both size and carbonate mass. Generally, supersaturation with respect to calcite and aragonite is observed throughout the entire Basin, which may be one of the reasons for overcalcification observed in coccolithophores in the eastern Mediterranean surface sediments. At the same time, however, effects that are ascribed to drastic increase in anthropogenic CO₂ and subsequently in surface water acidification can be possibly observed in the coccolith morphology in other parts of the Mediterranean.

BACKGROUND

The unique setting of the Mediterranean Sea makes it an excellent experimental setup to study and predict the impact of ocean acidification (OA). The surface water masses (i.e. where primary production and calcification is occurring) is characterized by steep W/E directed physico-chemical gradients, with increasing salinity, temperature, stratification and alkalinity towards the east. The generally oligotrophic to ultraoligotrophic offshore waters stand in contrast to eutrophication in certain coastal areas with coral and seagrass ecosystems.

In the Mediterranean region, drastic and rapid increase of anthropogenic greenhouse gas concentrations not only cause OA but dramatically increase heat stress risk, with the occurrence of hot extremes expected to increase by 200 to 500% throughout the region (Diffenbaugh *et al.*, 2007). The response of the highly adapted Mediterranean marine organisms (carbonate and non-carbonate) to this increased warming stress and to OA is poorly understood. Due to the short residence time in the Mediterranean Sea future changes will take place relatively fast. With the

rapid increase of anthropogenic CO₂ in the Mediterranean region, a larger absorption by seawater is expected, consequently a reduction in seawater pH and carbonate ion concentration is anticipated. Carbonate ions are a basic building block of skeletons and shells for a large number of marine organisms including corals, shellfish, and marine plankton.

CALCIFYING PLANKTONIC ORGANISMS

Coccolithophores are a major calcifying planktonic organism in the Mediterranean (and in the oceans in general) and a major producer of calcium carbonate. Coccoliths (micrometric calcite plates produced by coccolithophore algae) are major contributors of the carbonate flux and sedimentation to the deep sea (Milliman, 1993). They can also act as mineral ballast which plays a role in the export of particulate organic matter to the deep ocean (Ziveri *et al.*, 2007). Given that the density of carbonate is higher than opal and is more abundant than lithogenic material in the ocean (Klaas and Archer, 2002), a relation between organic carbon export and calcium carbonate is suggested. In addition, a positive correlation between relatively heavy coccolith carbonate mass, i.e. *Calcidiscus leptoporus* and *Helicosphaera carteri*, and organic carbon daily fluxes in a sediment trap from the North Atlantic shows their effectiveness as mineral ballast (Ziveri *et al.*, 2007; 2008).

There have been controversial results from culture experiments (Riebesell *et al.*, 2000; Langer *et al.*, 2006; Iglesias-Rodriguez *et al.*, 2008) on the impact of CO₂ increase on coccolithophore calcification. In general, since the Mediterranean is an area of extreme carbonate supersaturation, no signs of OA are expected there soon despite high anthropogenic carbon input. However a recent a workshop co-sponsored by the European Science Foundation (Euroclimate Program) and PAGES (Past Global Change) on atmospheric CO₂, ocean acidification and ecological changes in planktonic calcifying organisms presented unpublished results that showed otherwise, e.g. large changes in the thermohaline circulation from 2001 – 2006 influencing the uptake of anthropogenic carbon and thus seawater carbonate parameters, as well as first organismal response to the observed changes (Kiefer *et al.*, 2008; Ziveri *et al.*, 2008).

COCCOLITHOPHORE CARBONATE RESPONSE TO ENVIRONMENTAL CHANGES

The ESF-CRP MERF (Quaternary marine ecosystem response to fertilization: Mediterranean sapropel events and implications for marine carbon uptake) aims to detect the impact of changes in fertilization on marine productivity and carbonate response in the present and during times of natural Quaternary fertilization in the Mediterranean Sea. This enclosed Sea is presently characterized by low primary production but in the recent past changes in nutrient fluxes were occurring, modulated by monsoon cycles (Rossignol-Strick *et al.*, 1982). In the eastern Mediterranean these cycles are characterized by the deposition of organic rich layers (sapropels). Calcification in coccolithophores is influenced by several factors such as nutrient concentration (including micronutrients), carbonate chemistry of sea water (carbonate ions), temperature, and cell physiology.

MODERN MEDITERRANEAN

Our results showed that in the present Mediterranean, during times of high production and fertilization, the coccoliths are larger in size and more calcified than during the rest of the year. This is in accordance with the culturing experiments that have shown that coccoliths formed at higher growth rates are generally better calcified. However, when we compare *Emiliania huxleyi* (the most common living coccolithophore species) and its carbonate quota in surface sediment from the whole Mediterranean, heavier coccoliths are found in the eastern Mediterranean where nutrient concentration is in general much lower than in the western Mediterranean. A possible explanation of this pattern is that the calcification of *E. huxleyi* is strongly related to the carbonate saturation state. In the Gulf of Lions, a long-term decrease in lith weight of *E. huxleyi* is superimposed on the annual variability observed in the sediment trap series. This decrease is paralleled by a decrease of carbonate ions, probably due to the increase of anthropogenic CO₂ uptake from the atmosphere. In surface sediments, *E. huxleyi* presents a large variability in size, morphology and carbonate mass. We believe that this is partly related to the variable carbonate ion concentration. Whereas

weight distributions within *E. huxleyi* are very homogenous in the Western Mediterranean Sea, a lot more variability and in general heavier calcified liths can be observed in the Eastern Mediterranean where the carbonate saturation levels are generally higher (Schneider *et al.*, 2007).

SAPROPEL 1 AND CHANGE IN COCCOLITH AND FORAMINIFERA CARBONATE WEIGHT

Interestingly, in the recent past, during the deposition of most recent sapropel 1 (~9,000 to 5,000 years BP), a comparison of cores on a W-E transect has shown that *E. huxleyi* weight and size decreases (Principato *et al.*, 2006) in the Eastern Mediterranean, whereas it slightly increases in time equivalent series of the Western Mediterranean. Despite generally higher levels of productivity in the eastern Mediterranean, which should lead to a size and weight increase in *E. huxleyi* according to sediment trap and culture observations, we observed a size and weight decrease instead. This may be explained by (a) different fertilization mechanisms (Sr/Ca in coccolith calcite shows higher productivity during S1 in the eastern Mediterranean and no change during the time equivalent sediment in the western Mediterranean), or (b) by a different saturation state of bottom waters (influencing carbonate preservation) and carbonate ion concentration (influencing primary calcification) in the production layer (Schneider *et al.*, 2007). Theoretically faster growth rates induced by fertilization could enhance coccolith calcification during sapropel formation, however this is not observed. Therefore the change in size and weight during sapropel formation is most probably due to changes in the carbonate saturation state of the Eastern Mediterranean.

INORGANIC CARBONATE

In addition, coccolithophores and foraminifera from the eastern Mediterranean sediments can have inorganically precipitated calcite (diagenetic carbonate production) (Crudeli *et al.*, 2004). For coccolithophores *E. huxleyi* is the species most affected by this process. A possible explanation causing this very unusual process is the elevated sediment pore water alkalinity and saturation state. This overcalcification is absent during sapropel deposition.

FINAL REMARKS AND OPEN QUESTIONS

Recently published results on a Mediterranean site, naturally carbonated by submarine volcanoes, provide clues to the possible effects of high-CO₂ conditions on marine ecosystems (see Hall-Spencer and Rodolfo-Metalpa, this volume; Hall-Spencer *et al.*, 2008). In addition several electric power stations and other factories situated on the coast in many Mediterranean areas emit substantial quantities of greenhouse gases at the local level. These sites could serve as natural laboratories for OA studies.

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