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Impact of elevated CO₂ on shellfish calcification

Frédéric Gazeau,¹ Christophe Quiblier,¹ Jeroen M. Jansen,¹ Jean-Pierre Gattuso,^{2,3} Jack J. Middelburg,¹ and Carlo H. R. Heip¹

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[1] 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. Here, we demonstrate that the calcification rates of the edible mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) decline linearly with increasing pCO₂. Mussel and oyster calcification may decrease by 25 and 10%, respectively, by the end of the century, following the IPCC IS92a scenario (~740 ppmv in 2100). Moreover, mussels dissolve at pCO₂ values exceeding a threshold value of ~1800 ppmv. As these two species are important ecosystem engineers in coastal ecosystems and represent a large part of worldwide aquaculture production, the predicted decrease of calcification in response to ocean acidification will probably have an impact on coastal biodiversity and ecosystem functioning as well as potentially lead to significant economic loss.

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1. Introduction

[2] The Intergovernmental Panel on Climate Change (IPCC) predicts atmospheric CO₂ partial pressure (pCO₂) ranging from 490 to 1,250 ppmv in 2100, depending on the socio-economic scenario considered [Prentice *et al.*, 2001]. Because one third of anthropogenic CO₂ emissions has been stored in the oceans, ocean pH has already declined by 0.1 unit compared with pre-industrial 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 CO₂ and lower carbonate ion (CO₃²⁻) concentrations. The carbonate ion is one of the building blocks of calcium carbonate (CaCO₃) and changes in its ambient concentration can thus affect the ability of calcifying organisms to precipitate CaCO₃.

[3] Indeed, marine organisms such as coral reefs, foraminifera, coralline algae and mollusks can produce cal-

careous skeletons or shells following the simplified reaction:



As, at a constant salinity, calcium concentration is rather constant in the ocean, the calcification process mainly depends on the availability of CO₃²⁻. The calcium carbonate saturation state:

$$\Omega = \frac{[\text{CO}_3^{2-}][\text{Ca}^{2+}]}{K'_{sp}} \quad (2)$$

where K'_{sp} is the stoichiometric solubility product (dependent on temperature, salinity, pressure and on the considered mineral phase: calcite, aragonite or high-magnesian calcite) will therefore decrease although, the surface ocean will remain almost entirely supersaturated (Ω > 1) with respect to calcite and aragonite, the only exception being Ω_{aragonite} in cold waters [Orr *et al.*, 2005].

[4] Several experiments have shown a reduction of calcification and size at elevated pCO₂ in corals, coralline algae, coccolithophorids and foraminifera [Agegian, 1985; Bijma *et al.*, 1999; Leclercq *et al.*, 2000; Riebesell *et al.*, 2000; Langdon and Atkinson, 2005]. Few studies have investigated the detrimental effect of acidic waters on bivalves [Bamber, 1990; Michaelidis *et al.*, 2005; Berge *et al.*, 2006] and none investigated the response of calcification to pCO₂ levels within the range of values projected by IPCC.

2. Material and Methods

[5] Juvenile and adult specimen of *Mytilus edulis* and *Crassostrea gigas* were collected at low tide in Paulinapolder (Western Scheldt estuary) and near Yerseke (Eastern Scheldt estuary), respectively. In the laboratory, individuals were cleaned and stored in bags placed in situ in front of the laboratory (Eastern Scheldt). Experiments took place in May/June 2006 and July 2006 for mussels and oysters respectively. About 400 g of fresh weight (shell + tissue; g FW) of mussels and 1000 g FW of oysters were placed in two 10 L aquaria. Each aquarium was linked to a 10 L buffering tank as shown on Figure 1, in which CO₂-free air (outside air with CO₂ removed using soda lime) was bubbled. Water circulation between the tanks and the aquaria was performed by pumps with a capacity of 10 L min⁻¹. Aquaria and equilibration tanks were filled with water from the Eastern Scheldt (salinity of 30), 50% of the total volume was replaced every day. The temperature of the experimental room was maintained constant at 20°C. Bivalves were fed daily on a commercial mixture composed

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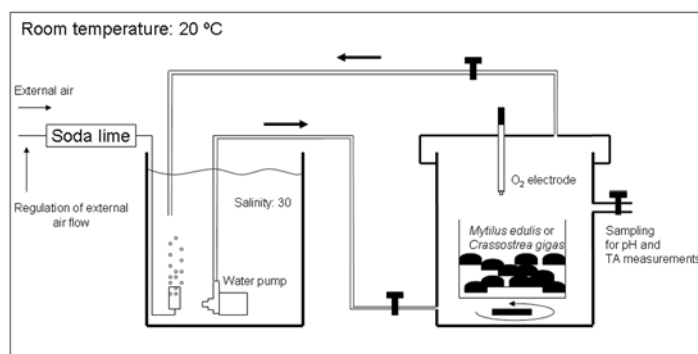


Figure 1. Experimental set-up used in this study. See text for details.

of *Isochrysis* (25%), *Palova* (20%), *Tetraselmis* (20%) and *Thalassiosira weissflogii* (35%; Shellfish diet 1800; Reed Mariculture).

[6] In the chambers, pCO₂ and O₂ concentrations increased and decreased, respectively, due to the respiration of the organisms. CO₂-free air bubbling was modulated to set the pCO₂ at the desired value and to keep oxygen concentrations near saturation. One tank was maintained at a pCO₂ of 600 ± 100 ppmv by strong bubbling (600 ppmv is the ambient average pCO₂ in the Eastern Scheldt during the periods of investigation; data not shown), while in the other set-up, air-bubbling was modulated to fix the pCO₂ at the desired level. Series of two or three incubations were performed daily by isolating the incubation chambers from contact with the equilibration chambers. Incubations lasted for 2 hours and were performed in parallel in the two experimental set-ups. At the start of the experiments, the first incubation served as a comparison between the two set-ups at approximately the same pCO₂ (~600–700 ppmv). Rates of calcification of both mussels and oysters were similar in the two tanks eliminating the possibility of a strong natural variability between the two incubated populations. A water sample (200 mL) was taken before and after each incubation to measure pH and total alkalinity (TA). pH was measured immediately after sampling using a combined electrode (Metrohm) calibrated daily with NBS buffers and TA was measured in triplicate within 1 day by Gran electro-titration on 10 mL GF/F filtered samples. pCO₂ was computed from pH and TA using the thermodynamic constants of *Mehrbach et al.* [1973] and a DOS-based CO₂ software (developed by Michel Frankignoulle). The saturation solubility products for calcite and aragonite were taken from *Morse et al.* [1980].

[7] Net calcification rates (G in mmol CaCO₃ g FW⁻¹ h⁻¹) were estimated using the alkalinity anomaly technique [*Smith and Key*, 1975] following the equation:

$$G = \frac{-\Delta TA}{2} \quad (3)$$

Where ΔTA is the variation of TA during the incubations in meq g FW⁻¹ h⁻¹. This technique is based on the fact that the precipitation of 1 mole of CaCO₃ consumes 2 moles of HCO₃⁻, therefore decreasing TA by 2 equivalents. Incubations of 2 h were sufficient to observe significant changes of TA (ΔTA values ranged from -74 to 22 $\mu\text{eq L}^{-1}$). During the incubations, respiration and calcification caused pH and

pCO₂ to decrease and increase, respectively (mean $\Delta\text{pH} = -0.22 \pm 0.05$ and mean $\Delta\text{pCO}_2 = 579 \pm 311$ ppmv). Therefore, we report pH and pCO₂ values averaged over the 2 h incubation period.

3. Results and Discussion

[8] The calcification rates of the edible mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) exhibit a strong decline as a function of decreasing pH, increasing pCO₂ and decreasing [CO₃²⁻] (Figures 2a, 2b, and 2c). Excretion of ammonium resulting from the catabolism of organic nitrogen potentially causes an increase in TA by 1 equivalent per mole. *Bayne and Widdows* [1978] and *Okumus and Stirling* [1994] reported ammonium excretion rates for *Mytilus edulis* of ~15 μg per g of tissue dry weight. Moreover, *Michaelidis et al.* [2005] reported an ammonium excretion increase of ~150% under hypercapnia conditions (pH = 7.3). Based on their results, a ΔTA increase of ~1 $\mu\text{eq kg}^{-1} \text{h}^{-1}$ between low and high pCO₂ conditions can be expected (assuming a tissue dry weight: FW ratio of 5%) which is insignificant with regard to the ΔTA variations observed in the present study (difference of ~50 $\mu\text{eq kg}^{-1} \text{h}^{-1}$ between low and high pCO₂ conditions). Hypoxia and hypercapnia can lead to a metabolic stress for the organisms. In our study, oxygen concentrations were always kept near saturation values at the start of the incubations and respiration rates (measured on six occasions with pCO₂ ranging from 700 to 2000 ppmv; data not shown) did not reveal any significant effect of hypercapnia on this process. Results clearly highlight a dependency of calcification rates on pCO₂ and [CO₃²⁻] values for both species, although oysters appeared to be far less sensitive than mussels as shown by the lower slope of the lines between either pCO₂ or [CO₃²⁻] and net calcification. This can be partly explained by their different shell mineralogy, as the shell of *Crassostrea gigas* is mainly composed of calcite [*Stenzel*, 1963], while the shell of *Mytilus edulis* can contain up to 83% of aragonite [*Hubbard et al.*, 1981]. Tables 1 and 2 list the parameters measured during each incubation (pH and TA), computed pCO₂ and CO₃²⁻ concentrations, as well as the computed calcite and aragonite saturation states. During the mussel experiments, the water was never undersaturated with regard to calcite whereas at pCO₂ values higher than ~2000 ppmv (assuming TA = 2.5 meq kg⁻¹, S = 30 and T = 20°C), dissolution of aragonite might thermodynamically have occurred ($\Omega < 1$).

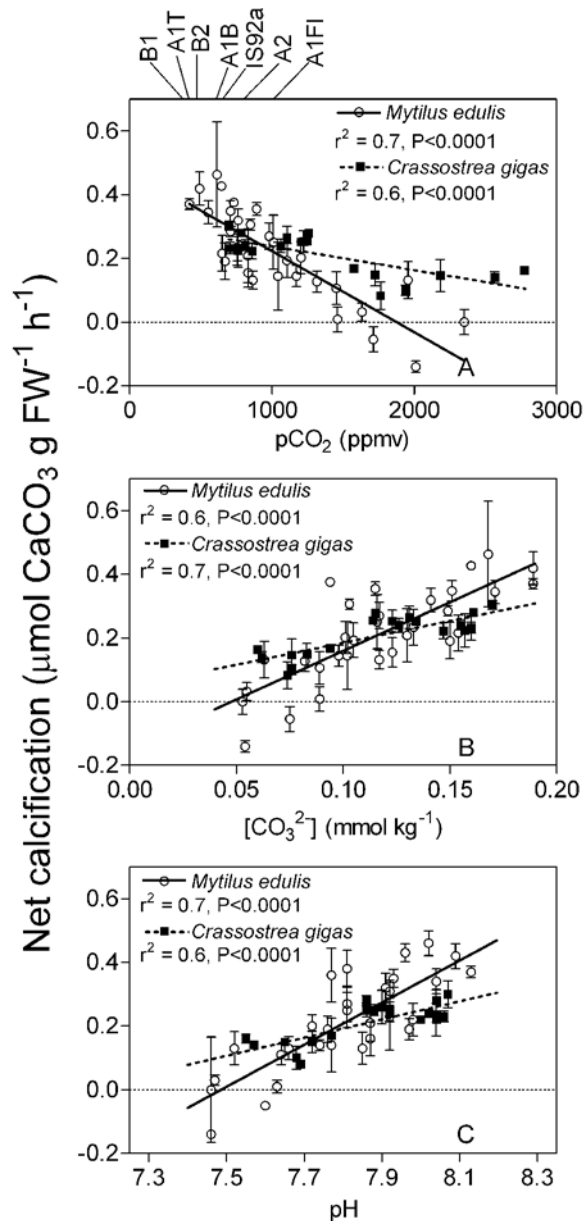


Figure 2. Relationships between mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) calcification per unit fresh weight and (a) partial pressure of CO₂ (pCO₂), (b) carbonate ions concentration ([CO₃²⁻]) and (c) pH. Errors bars represent the standard deviations calculated from the triplicate measurements of TA. On the upper panel, corresponding atmospheric pCO₂ values predicted by several IPCC socio-economic scenarios for the year 2100 are indicated. The lines represent linear fits; the coefficient of determination and its statistical significance are indicated.

Mussels dissolved at pCO₂ values exceeding a threshold value of ~1800 ppmv. TA values were significantly higher during the oyster experiment and even at the highest pCO₂ values, the water was never undersaturated with regard to aragonite and calcite. Although in these two experiments, both aragonite and calcite saturation states were, most of the time, above one, a clear impact of increasing pCO₂ and decreasing CO₃²⁻ concentrations on net calcification was highlighted for these two species. Previous studies reported

a decline of the calcification rate of benthic molluscs at pH values often lower than those expected in the present century [Bamber, 1990; Michaelidis et al., 2005; Berge et al., 2006]. They showed that these organisms were capable of maintaining a constant internal pH by decreasing their metabolic rates and/or dissolving their shell; the shell acting then as a source of CO₃²⁻. Berge et al. [2006] showed that the growth of *Mytilus edulis* at pH levels of 7.4 and 7.6 was not significantly different from growth at normal pH 8.1. The apparently contradictory results between our study and Berge et al. [2006] can be explained in a number of non-exclusive ways. First, it is obvious that the incubation times used in our study did not allow any potential adaptation by the organisms in comparison to the 45 days incubation period of 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 our study. 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 the shell thickness, and consequent decrease of shell weight is very likely [Bamber, 1990]. The alkalinity anomaly technique is much more sensitive and appropriate for relatively short term studies

Table 1. Average pH (NBS Scale), Total Alkalinity (TA in meq kg⁻¹), Partial Pressure of CO₂ (pCO₂ in ppmv), Carbonate Ion Concentrations (CO₃²⁻ in mmol kg⁻¹) and Saturation States of the Water Regarding Aragonite and Calcite Minerals (Ω_{aragonite} and Ω_{calcite} Respectively), During the Two Hour Incubations for *Mytilus Edulis* (Salinity: 30; 20°C)^a

pH	TA	pCO ₂	[CO ₃ ²⁻]	Ω _{aragonite}	Ω _{calcite}	G
8.13	2.421	421	0.189	3.4	6.3	0.37
8.09	2.573	493	0.189	3.4	6.3	0.42
8.04	2.537	552	0.171	3.1	5.7	0.34
8.02	2.636	614	0.168	3.0	5.6	0.46
7.96	2.569	645	0.160	2.9	5.4	0.43
7.98	2.556	651	0.154	2.8	5.2	0.22
7.97	2.556	671	0.150	2.7	5.0	0.19
7.93	2.590	709	0.151	2.7	5.2	0.35
7.81	2.008	733	0.094	1.7	3.9	0.38
7.92	2.515	756	0.133	2.4	4.5	0.23
7.92	2.272	849	0.103	1.9	3.4	0.31
7.91	2.588	763	0.141	2.5	4.7	0.32
7.87	2.562	831	0.130	2.3	4.4	0.21
7.87	2.494	832	0.123	2.2	4.1	0.16
7.85	2.473	865	0.117	2.1	3.9	0.13
7.77	2.383	893	0.115	2.1	3.9	0.36
7.81	2.592	981	0.117	2.1	3.9	0.27
7.81	2.626	1010	0.116	2.1	3.9	0.25
7.77	2.482	1043	0.102	1.8	3.4	0.14
7.76	2.581	1106	0.105	1.9	3.5	0.19
7.74	2.565	1172	0.098	1.8	3.3	0.14
7.72	2.607	1204	0.101	1.8	3.4	0.20
7.66	2.417	1315	0.082	1.5	2.7	0.13
7.64	2.641	1453	0.089	1.6	3.0	0.11
7.63	2.633	1461	0.089	1.6	3.0	0.01
7.47	2.091	1632	0.055	1.0	1.9	0.03
7.60	2.625	1713	0.075	1.3	2.5	-0.05
7.52	2.534	1956	0.063	1.1	2.1	0.13
7.46	2.344	2010	0.054	1.0	1.8	-0.14
7.46	2.546	2351	0.053	1.0	1.8	0.00

^aCalcification rates (G in mmol CaCO₃ g FW⁻¹ h⁻¹) are also presented.

Table 2. Average pH (NBS Scale), Total Alkalinity (TA in meq kg⁻¹), Partial Pressure of CO₂ (pCO₂ in ppmv), Carbonate Ion Concentrations (CO₃²⁻ in mmol kg⁻¹) and Saturation States of the Water Regarding Aragonite and Calcite Minerals ($\Omega_{\text{aragonite}}$ and Ω_{calcite} Respectively), During the Two Hour Incubations of *Crassostrea Gigas* (Salinity: 30; 20°C)^a

pH	TA	pCO ₂	[CO ₃ ²⁻]	$\Omega_{\text{aragonite}}$	Ω_{calcite}	G
8.07	2.740	698	0.170	3.1	5.7	0.30
8.06	2.645	705	0.160	2.9	5.3	0.23
8.04	2.742	761	0.160	2.9	5.4	0.23
8.04	2.742	762	0.157	2.8	5.3	0.22
8.04	2.727	782	0.161	2.9	5.4	0.28
8.02	2.722	811	0.155	2.8	5.2	0.24
8.00	2.788	861	0.147	2.7	4.9	0.22
7.92	2.710	1063	0.126	2.3	4.2	0.24
7.90	2.968	1106	0.131	2.4	4.4	0.26
7.92	2.811	1206	0.134	2.4	4.5	0.25
7.88	2.892	1222	0.123	2.2	4.1	0.25
7.86	2.684	1247	0.114	2.1	3.8	0.25
7.86	2.779	1258	0.115	2.1	3.8	0.28
7.77	2.694	1575	0.094	1.7	3.1	0.17
7.72	2.667	1724	0.083	1.1	2.0	0.15
7.69	2.659	1762	0.074	1.3	2.5	0.08
7.68	2.695	1938	0.076	1.4	2.5	0.10
7.65	2.785	2183	0.076	1.4	2.5	0.15
7.57	2.816	2564	0.062	1.1	2.1	0.14
7.55	2.736	2774	0.060	1.1	2.0	0.16

^aCalcification rates (G in mmol CaCO₃ g FW⁻¹ h⁻¹) are also presented.

(1 month or less) during which increments of length and weight are difficult to measure. It is also important to note that, in our study, pCO₂ and pH strongly increased and decreased respectively during the 2 h incubations. Future studies should test the possibility of using shorter incubation times and/or smaller biomass to limit this disturbance. More results on shell growth of these species under expected marine pH conditions by 2100 are therefore strongly needed. Following our results, with regard to calcification rates estimated from the relationships for a pCO₂ of 380 ppmv, mussel and oyster calcification may decrease by 25 and 10% respectively by the end of the century, following the IPCC IS92a scenario (~740 ppmv in 2100).

[9] Since Broecker and Takahashi [1966] suggested a dependency of calcification rates on CaCO₃ saturation state in the Bahama Banks, numerous experimental studies have investigated the effect of an increase of pCO₂ (decrease of CO₃²⁻) on the calcification rates of calcareous organisms. Langdon and Atkinson [2005] reviewed projected declines of coral calcification for a doubling atmospheric pCO₂ scenario and reported a wide range (1–80%). Moreover, with regard to heterotrophic organisms such as mollusks, a concomitant increase in seawater temperature will hypothetically lead to an increase of respiration rates and therefore accentuate the effect of increasing atmospheric pCO₂. Recently, Langer *et al.* [2006] observed that the effect of increasing CO₂ on coccolithophorids differs from one species to another and suggested that the notion of a linear relationship of calcification with the carbonate ion concentration and CaCO₃ saturation state has to be reconsidered. More importantly, based on the analysis of sediment cores covering a period from the last glacial maximum to preindustrial times, they showed that some species are certainly able to adapt their calcification mechanism to the change in carbonate chemistry. Although this hypothesis may be true for fast-growing and genetically diverse

species, this still has to be investigated for higher organisms.

[10] With an average annual increase of 7.9% over the last 30 years, global shellfish production reached 11.7 million tons (Total fresh weight = soft tissue + shell) in 2002, corresponding to a commercial value of US\$10.5 billion [Food and Agriculture Organization, 2004]. The Pacific oyster (*Crassostrea gigas*) was the most cultivated species in 2002 with a volume of 4.2 millions tons or 10.8% of the total world aquaculture production while mussel production represented 1.4 million tons (3.6% of total production). The predicted decrease of calcification in response to ocean acidification will most certainly cause a significant economic loss. Besides these direct shellfish harvesting losses, there are also indirect ecosystem services losses which are more difficult to quantify. Mussels and oysters are ecosystem engineers governing energy and nutrient flows in coastal ecosystems, providing habitats for many benthic organisms and constitute important resources for birds [Gutiérrez *et al.*, 2003]. Any future decline of these species may thus have major consequences for coastal biodiversity and ecosystem functioning and services. Juvenile bivalves are even more sensitive to ocean acidification and their high mortality rates have been already linked to calcium carbonate dissolution [Green *et al.*, 2004].

[11] In order to accurately assess socio-economic and ecological impacts of ocean acidification, it is urgent to investigate the adaptive response of calcareous organisms to long-term CO₂ enrichment and the interactive effect of the concomitant increase in seawater temperature. Although this preliminary study highlighted the negative effect of seawater acidification on calcification by benthic mollusks under projected atmospheric CO₂ rising scenarios by the end of this century, longer-term studies still need to be achieved in order to take into account a potential adaptation of the organisms to low pH environments. Finally, responses of other economically important species such as clams and cockles need to be investigated as impact of elevated pCO₂ on calcification is tightly linked to the shell mineralogy of the considered organism.

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