

Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*)

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Abstract. Thecosome pteropods (shelled pelagic molluscs) can play an important role in the food web of various ecosystems and play a key role in the cycling of carbon and carbonate. Since they harbor an aragonitic shell, they could be very sensitive to ocean acidification driven by the increase of anthropogenic CO₂ emissions. The impact of changes in the carbonate chemistry was investigated on *Limacina helicina*, a key species of Arctic ecosystems. Pteropods were kept in culture under controlled pH conditions corresponding to pCO₂ levels of 350 and 760 μatm. Calcification was estimated using a fluorochrome and the radioisotope ⁴⁵Ca. It exhibits a 28% decrease at the pH value expected for 2100 compared to the present pH value. This result supports the concern for the future of pteropods in a high-CO₂ world, as well as of those species dependent upon them as a food resource. A decline of their populations would likely cause dramatic changes to the structure, function and services of polar ecosystems.

1 Introduction

The oceans have absorbed about one third of total anthropogenic CO₂ emissions since 1800 (Sabine et al., 2004). Although this uptake of greenhouse gases limits global warming, it also causes profound changes in the chemistry of seawater such as a decrease of pH referred to as “ocean acidification”. Global mean surface ocean pH has decreased by about 0.1 unit since the end of 18th century and, accord-

ing to model projections, will decrease by another 0.3 unit by the end of the present century (Caldeira and Wickett, 2003). Such pH decrease will coincide with a decrease in the carbonate ion concentration of 55% (Brewer, 1997) which could severely impact most calcifying organisms such as corals and coralline algae (Gattuso et al., 1998; Langdon and Atkinson, 2005), commercial molluscs (Gazeau et al., 2007) or phytoplankton (Riebesell et al., 2000). Effects of ocean acidification are already detectable, for example, since the end of 18th century a 30–35% decline of Antarctic foraminifera shell weights has been reported (Moy et al., 2009).

Pteropods are pelagic molluscs that are highly specialized for life in the open ocean. They are commonly referred to as “sea butterflies”, due to the evolution of their gastropod foot into wing-like parapodia that allow them a pelagic existence (Lalli and Gilmer, 1989). They filter-feed and produce large mucus webs for collecting mostly phytoplankton but also small zooplankton or their juveniles (Gilmer and Harbison, 1986; Harbison and Gilmer, 1992; Gannefors et al., 2005). The “shelled pteropod” species produce a calcium carbonate shell; the only shelled pteropod in Arctic waters is *Limacina helicina*, a species that can occur in high densities in both Arctic and Southern Ocean. *Limacina helicina* is also an important component of marine food webs. For example in the Southern Ocean it is frequently very abundant (up to 2681 ind m⁻³) and is a major dietary component for zooplankton and higher predators such as herring, salmon, whale and birds (Hunt et al., 2008; Karnovsky et al., 2008). Shelled pteropods may also play a geochemical role in the oceans, as significant contributors to the export of carbonate (order 10% of the global CaCO₃ flux, Berner and Honjo, 1981) and carbon to the deep ocean (Collier et al., 2000).



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There is great concern about the potential impact of the consequences of ocean acidification on high-latitude organisms and ecosystems. Models predict that the surface waters of the polar oceans will be the first to become undersaturated with respect to aragonite in 2050 in the Southern Ocean (Orr et al., 2005; McNeil and Matear, 2007) and as early as 2016 in the Arctic Ocean (Steinacher et al., 2009). Pteropod shells are made of aragonite, a metastable form of calcium carbonate more soluble than calcite in seawater (Mucci, 1983). These key organisms, are therefore expected to be highly sensitive to ocean acidification.

The rate of calcification for four pteropod species (none of them *Limacina helicina*) were reported by Fabry (1990) but despite their important role in the food web as well as in the cycling of carbon and calcium carbonate, not a single study has investigated their response to ocean acidification. Here we report on the first measurements of calcification of a pteropod in perturbation experiments where the CO_2 partial pressure ($p\text{CO}_2$) and temperature were controlled. The rate of calcification of *Limacina helicina* was measured under 1990 ($p\text{CO}_2=350 \mu\text{atm}$) and end-of-century conditions ($p\text{CO}_2=765 \mu\text{atm}$) consistent with Intergovernmental Panel on Climate Change (IPCC) projections.

2 Materials and methods

2.1 Sampling

Pteropods were collected in Kongsfjorden, Svalbard (Fig. 1) during the period 19 May to 8 June 2008. West Spitsbergen is influenced by a branch of the North Atlantic Current that carries warm and saline waters. Kongsfjorden is an open fjord, submitted to the influence of the Atlantic and Arctic waters (Svendsen et al., 2002).

Jars mounted on sticks were used to collect animals swimming near the surface, the bottom depth was less than 250 m. This method avoided the stress and damage to the body or the shell which can occur with nets. Pteropods were immediately transported to the Kings Bay Marine Laboratory in Ny-Ålesund, and maintained in 51 beakers under controlled conditions. The seawater used in the experiments was pumped at 80 m and filtered on $20 \mu\text{m}$ filters. The beakers were temperature-controlled at 5°C and gently stirred using slowly rotating plastic paddles (10 rpm).

2.2 Measurement of the carbonate chemistry

Seawater pH was measured within one hour of collection on a field sample collected 30 May. It was also measured in the experimental beakers, using a pH meter (Metrohm, 826 pH mobile) with a glass electrode (Metrohm, electrode plus) calibrated every second day on the total scale using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 35.0 (Dickson et al., 2007). Total alkalinity (TA) was measured on a field sample collected on 30 May as

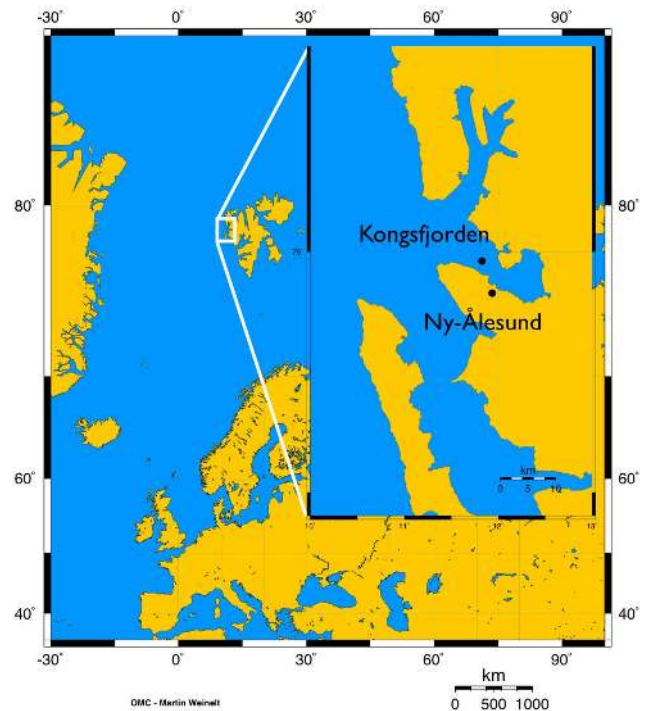


Fig. 1. Location of the field experiment. Pteropods were collected in the Kongsfjorden. Experiments were carried out at the marine laboratory in Ny-Ålesund ($78^\circ55' \text{ N } 11^\circ56' \text{ E}$).

well as in experimental samples which were filtered, poisoned with HgCl_2 and stored in a cool dark place pending measurement. It was determined potentiometrically using a home-made titrator built with a Metrohm pH electrode and a 665 Dosimat titrator. Measurements were carried out on 20 ml samples at 25°C and TA was calculated using a Gran function applied to the pH values ranging from 3.5 to 3.0 as described by Dickson et al. (2007). Titrations of a total alkalinity standard provided by A. G. Dickson (batch 80) were within $0.7 \mu\text{mol kg}^{-1}$ of the nominal value (standard deviation= $2.6 \mu\text{mol kg}^{-1}$; $n=8$). The concentration of dissolved inorganic carbon (DIC) and the saturation state of aragonite (Ω_a) were determined from pH_T and total alkalinity using the R package *seacarb* (Proye and Gattuso, 2003).

2.3 Calcein staining

A batch of 50 pteropods, freshly collected and transferred to the laboratory, were stained in a calcein bath for 1 h (final concentration: 50 mg l^{-1}). The animals were then rinsed by successive transfers in unstained seawater. They were maintained for 5 d in 51 beakers under controlled conditions of pH and temperature. pH was controlled at either 7.8 or 8.09 using a continuous pH-stat system (IKS, Karlsbad) that bubbled either CO_2 -free air or pure CO_2 depending on the desired pH value. Sea water for TA measurements was collected daily. Upon completion of the incubation, only active

(or swimming) pteropods were sampled and observations made under UV-epifluorescence using a Leica DM2000 microscope.

2.4 ^{45}Ca uptake

Freshly collected pteropods were incubated with $^{45}\text{CaCl}_2$ (16 Bq ml^{-1}) in four 5 l beakers at two pH levels. Two groups comprised 18 animals each and served for time point 0 (actually about 1 min exposure to ^{45}Ca) while the other groups comprised 54 animals each and served for time points 2, 4 and 6 h. pH_T was maintained at 7.78 (low pH) and 8.09 (normal pH) by continuous, gentle bubbling of air or CO_2 -enriched air generated by a gas mixing pump (Wösthoff, Bochum).

Six animals were sampled in triplicate at times 0, 2, 4 and 6 h, rinsed with unlabelled seawater, gently dried with a tissue to remove seawater, and weighed ($\pm 0.1\text{ mg}$). Shells were dissolved with 0.5 N HNO_3 and soft tissues were tissue-dried and weighed. The solutions were then neutralized using 2 N NaOH and 10 ml of scintillation liquid (Ultima Gold, PerkinElmer) added. Counting was performed with a Packard scintillation counter. An identical protocol was used with pteropods killed by HgCl_2 prior to incubation in order to estimate the non-biological incorporation of ^{45}Ca in the shell. The amount of CaCO_3 incorporated in the shell was:

$$Q_{\text{CaCO}_3} = \frac{1}{A_S \cdot M_{\text{Ca}} \cdot P} \left(\frac{R_l}{W_l} - \frac{R_d}{W_d} \right) \quad (1)$$

where Q_{CaCO_3} is the calcium carbonate precipitated ($\mu\text{mol (g wet weight)}^{-1}$), A_S is the specific activity of ^{45}Ca (Bq mg^{-1}), M_{Ca} is the molar mass of ^{45}Ca (g mol^{-1}), P is the ratio of radioactive vs. non radioactive calcium (estimated using its relationship with salinity, Dickson et al., 2007), R_l and R_d (Bq) are the radioactivity measured in the live and dead pteropods shells, W_l and W_d are the tissue wet weight (g) of the live and dead pteropods. Rates of calcification were derived by regressing Q_{CaCO_3} against time.

3 Results

Only one water sample was collected in the fjord; the values reported therefore do not provide information on the diurnal or geographic distribution of the parameters of the carbonate chemistry. Nevertheless, the parameters measured or estimated in the fjord are very close to those measured in the “normal pH” experimental condition (Table 1). The experimental values of Ω_a were 1.9 and 1.0 in the experimental conditions at pH_T 8.09 and pH_T 7.78, respectively.

The size range, based on the maximum diameter of the shell, was between 5 and 10 mm in the two experiments. The rate of survival after the 5-days period following the calcein staining was 100%. The survivorship was obtained by counting pteropods under a binocular at the completion

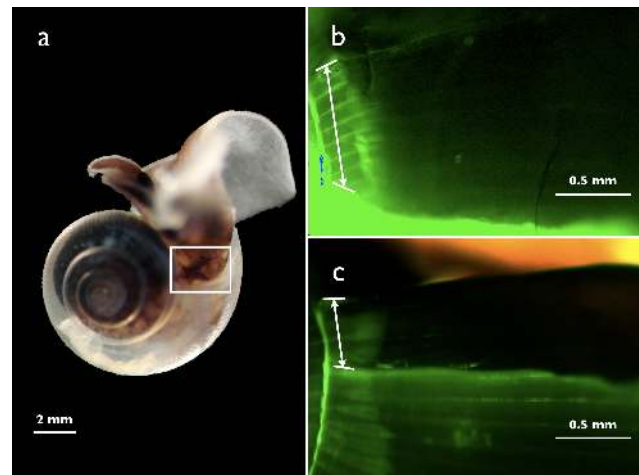


Fig. 2. Representative example of live Arctic *Limacina helicina* (a) stained with calcein and subsequently maintained at pH_T 8.09 (b) and 7.8 (c). Most calcification occurs near the shell opening (white rectangle). The arrow indicates the 5 days linear extent of the shell.

of the 5 day experiment. Although all pteropods were alive, only 30% were active swimmers. The others stayed at the bottom of the beaker and exhibited little activity. The fluoro-chrome calcein was successfully incorporated in the shells of living pteropods (Fig. 2). The dark part of the shell corresponds to the calcification that occurred over the 5 days following staining and demonstrates that the organisms were viable and performed calcification in both culture conditions. Qualitative examination of stained pteropods suggests that the linear extension of the shell during the 5-days period following staining was lower in individuals maintained at the low pH condition than in those maintained at higher pH. Too few organisms were photographed to provide statistically robust quantitative estimates of shell extension. The linear extensions measured are provided as a supplementary information (see <http://www.biogeosciences.net/6/1877/2009/bg-6-1877-2009-supplement.zip>).

The non-biological adsorption of ^{45}Ca on the shell was 0.25 ± 0.029 (mean \pm SD, $n=10$, $p < 0.001$) $\mu\text{mol CaCO}_3$ ($\text{g wet weight}^{-1} \text{ h}^{-1}$). The calcification rate measured during the 6 h incubation at pH_T 8.09 (corresponding to a pCO_2 of $350\text{ }\mu\text{atm}$) was 0.36 ± 0.027 ($n=10$, $p < 0.001$) $\mu\text{mol CaCO}_3$ ($\text{g wet weight}^{-1} \text{ h}^{-1}$) (Fig. 3). At pH_T 7.78 (corresponding to a pCO_2 of $765\text{ }\mu\text{atm}$), the calcification rate was 0.26 ± 0.018 ($n=10$, $p < 0.001$) $\mu\text{mol CaCO}_3$ ($\text{g wet weight}^{-1} \text{ h}^{-1}$). The coefficients of determination (R^2) of the rates of calcification regressed against time were 0.96 at pH_T 8.09 and 0.95 at pH_T 7.78. A comparison t -test confirmed that the slopes were statistically different ($t=3.0$, $p=0.02$, 9 df). The rate of calcification was 28% lower in the high than in the control CO_2 level. The difference in calcification between the two pH conditions was not significant during the first 2 h, significant during the first 4 h ($p=0.05$), and not significant during the last 2 h (hours 4 to 6) but no

Table 1. Parameters of the carbonate chemistry in the two experimental conditions and in the natural condition (middle of the fjord). The concentration of dissolved inorganic carbon (DIC), partial pressure of CO₂ (*p*CO₂) and the saturation state of aragonite (Ω_a) were derived from pH_T, total alkalinity, salinity and temperature.

Conditions	pH _T	Total alkalinity ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	<i>p</i> CO ₂ (μatm)	Ω_a	Salinity	Temperature (°C)
Normal pH (year 1990)	8.09	2298	2131	350	1.90	34.8	5
Low pH (year 2100)	7.78	2295	2237	765	1.00	34.8	5
Fjord (field measurement)	8.12	2312	2148	320	1.81	34.9	2.2

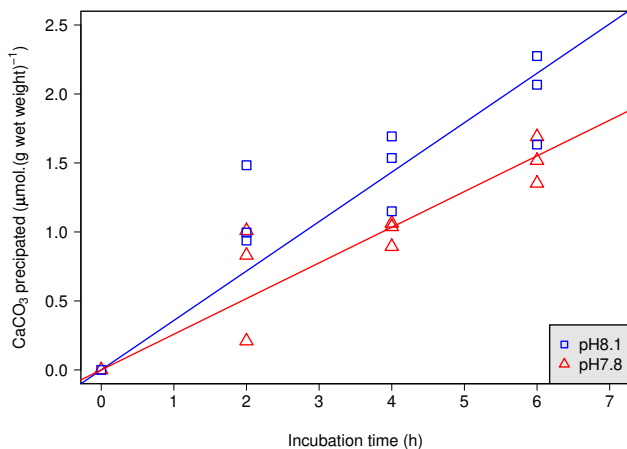


Fig. 3. Calcium carbonate precipitated as a function of time based on ⁴⁵Ca uptake.

significant change was found, at each pH, between the first 4 h and the last 2 h of the experiment.

4 Discussion

The Arctic Ocean is particularly vulnerable to the impact of seawater chemistry changes associated with ocean acidification. Models show that calcium carbonate undersaturation with respect to aragonite is expected as early as 2016 (Steinacher et al., 2009). Once the *p*CO₂ values reaches 409 μatm , for at least one month of the year, the entire water column will become undersaturated with respect to aragonite ($\Omega_a \leq 1$) in as much as 10% of the Arctic Ocean. These low saturation zones will be associated with increased melting of ice and freshwater inputs (Steinacher et al., 2009). Surface waters at our sampling location in Spitsbergen do not seem to be as vulnerable to aragonite undersaturation, as the aragonite undersaturation threshold ($\Omega_a=1$) was reached by bubbling the fjord water with air having a *p*CO₂ of 765 μatm .

At least two reasons could explain this difference. First, the sampling region is influenced by Atlantic water with high total alkalinity. Also, the experimental seawater pumped at 80 m was most likely a “Transformed Atlantic Water” (Cottier et al., 2005), less vulnerable to aragonite undersaturation than Arctic waters. Second, the experiments were carried out at a temperature of 5°C but lower temperatures occur in high Arctic waters further decreasing the solubility of aragonite.

This study is the first to provide both qualitative and quantitative evidence that increased ocean acidification affects calcification rates in pteropods. Calcein was previously used in studies of calcification in mussels (Reusch, 1998) and foraminifera (Erez, 2003). We show that calcein staining can also be used to investigate calcification in pteropods. It allowed comparison of the linear extension of the shells, which was lower at a pH_T of 7.8 than at a pH_T of 8.1. It must be pointed out, however, that statistical inference is not possible due to the small size of the data set and that this result remains to be confirmed in future studies. It is recommended to standardize measurements of linear extension as its value significantly depends on the location of its measurement. Either the minimum extension (as shown in Fig. 2) or the maximum extension in the middle of the shell edge are the less subjective ways to proceed. Note also that linear extension is not always a good indicator of the rate of calcification, because it does not necessarily correlate with shell thickness and/or density.

The qualitative result obtained with calcein staining was confirmed by quantitative measurements of calcification in a ⁴⁵Ca uptake experiment. Previous studies have shown a rapid decrease of calcification in pteropods maintained for several hours in laboratory conditions (Fabry, 1990). In the present study, this effect was partly avoided by determining calcification rates on freshly collected animals, by minimizing the sampling stress, and by limiting the incubation time to 6 h. The decrease in calcification at the lower pH mostly occurred during the first 4 h of the experiment as calcification was similar in both pH conditions between 4 and 6 h. However, the differences in calcification between the periods 0 to

4 h and 4 to 6 h, within each pH conditions, are not statistically significant. We therefore discuss the rates of calcification obtained during the entire experiment. Future studies are needed to identify possible short-term, acute responses as well as possible acclimation. This will require the use of a larger number of individuals in order to increase statistical power and as well as to improve the maintenance of live pteropods to conduct perturbation experiments. The 28% decline of calcification in the lower pH condition is within the range reported for other calcifying organisms such as corals (Langdon and Atkinson, 2005). Previous work on benthic molluscs also showed a decrease in calcification with decreasing pH (Gazeau et al., 2007). *L. helicina* does appear to be well-adapted to low aragonite saturation conditions, however, as the ^{45}Ca experiments demonstrate that it can maintain a positive net calcification at the aragonite saturation threshold ($\Omega_a=1.0$). In contrast, some organisms such as corals are unable to calcify at $\Omega_a=1.0$ (Langdon and Atkinson, 2005).

In the coming years, one may begin to witness a shift towards a negative balance between calcification and dissolution. Indeed, even if organisms can maintain calcification in undersaturated conditions, ocean acidification will increase the rate of dissolution of shells and skeletons. Pteropods are able to survive for 2 days in waters undersaturated with respect to aragonite but their shells show dissolution marks, casting additional doubt on their ability to achieve a positive balance between precipitation and dissolution of calcium carbonate (Orr et al., 2005). Furthermore, pteropods are vertical migrators that can cover several hundreds of meters per day (Wormuth, 1981). With the shoaling of the aragonite saturation state (Orr et al., 2005), pteropods might be exposed to increasingly corrosive waters during their daily migrations.

This work opens the way to new experiments on the effect of climate change on pteropods and highlights the critical need of new techniques enabling the maintenance of pteropods in the long term (several months). This would allow to avoid any stress related to sampling and to investigate the physiology plasticity and acclimation processes of pteropods. In the present study, temperature could not be maintained at its in situ level and experiments were carried out at a temperature 2.8°C higher than in situ. The lower rate of calcification found at the lower pH could therefore be the result of a response to both elevated $p\text{CO}_2$ and temperature. Future experiments will need to take into account the combined effects of elevated $p\text{CO}_2$ and temperature as it has been shown that the rate of calcification of the scleractinian coral *Stylophora pistillata* decreases by 50% under conditions of elevated temperature (28°C) and high $p\text{CO}_2$ (760 μatm) whereas no effect was found when only $p\text{CO}_2$ is elevated CO_2 ($p\text{CO}_2=760 \mu\text{atm}$; $t=25^\circ\text{C}$; Reynaud et al., 2003).

The results of this study support the concern for the future of pteropods in a high- CO_2 world, as well as of those species dependent upon them as a food resource. A decline

of their populations would likely cause dramatic changes to the structure, function and services of polar ecosystems.

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