NOTE

Effects of ocean acidification on overwintering juvenile Arctic pteropods *Limacina helicina*

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ABSTRACT: Pteropods are planktonic mollusks that play an important role in the food web of various ecosystems, particularly at high latitudes. Because they produce an aragonitic shell, pteropods are expected to be very sensitive to ocean acidification driven by anthropogenic CO_2 emissions. The effect of ocean acidification was investigated using juveniles of the Arctic pteropod *Limacina helicina* from the Canada Basin of the Arctic Ocean. The animals were maintained in 3 controlled pH conditions (total scale pH [pH_T] $\approx$ 8.05, 7.90 or 7.75) for 8 d, and their mortality and the linear extension of their shell were monitored. The pH did not impact the mortality rate, but the linear extension of the shell decreased as a function of declining pH. Surprisingly, the pteropods were still able to extend their shell at an aragonite saturation state as low as 0.6. Nevertheless, dissolution marks were visible on the whole shell, indicating that calcium carbonate dissolution had also occurred, casting doubts on the ability of the pteropods to maintain a positive balance between precipitation and dissolution of calcium carbonate under corrosive conditions.

KEY WORDS: Ocean acidification · Arctic · Pteropod · Shell growth

INTRODUCTION

Pteropods are pelagic mollusks that play an important role in the food web of extensive oceanic regions, particularly at high latitudes, where they are a major dietary component for zooplankton and higher predators, such as herring, salmon, whales and birds (Hunt et al. 2008). They have an aragonitic shell that acts as ballast, facilitating vertical migration, and protection against their environment (Lalli & Gilmer 1989). Pteropods are also involved in biogeochemical cycles, to which they are important contributors to the export of calcium carbonate and carbon to the deep ocean (e.g. Honjo 2004, Gangstø et al. 2008).

Pteropods are a current subject of concern due to their sensitivity to ocean acidification, caused by the dissolution of one-fourth of anthropogenic CO_2 emissions into the oceans. This uptake limited the increase in the concentration of atmospheric CO_2 by 55 ppm (Sabine et al. 2004). However, when CO_2 dissolves in seawater, it reacts with H_2O to form carbonic acid and reduces the pH of the ocean. Since pre-industrial time, the surface ocean pH has declined by 0.1 unit, and a further decline of 0.3 to 0.4 units is expected by the end of the century (Orr 2011). Among other changes, declining pH also causes a decrease in the level of carbonate ions (CO_3^{2-}) and its associated calcium carbonate saturation state (Ω). Studies have mainly demonstrated the

deleterious effect of ocean acidification on many calcifiers, such as coccolithophores (e.g. Riebesell et al. 2000), corals and coralline algae (e.g. Anthony et al. 2008, Martin & Gattuso 2009) and mollusks (e.g. Michaelidis et al. 2005, Gazeau et al. 2010). However, some studies reported contradictory results, such as no effects or positive effects of ocean acidification on several calcifiers (e.g. Iglesias-Rodriguez et al. 2008, Ries et al. 2009). The possible causes and consequences of such contradictory results were recently examined by Gattuso & Riebesell (2011).

Experiments carried out in Spitsbergen show a negative impact of ocean acidification on the calcification of both adults (Comeau et al. 2009, 2010a) and early juveniles of *Limacina helicina* (Lischka et al. 2011). Negative effects of CO_2 on juveniles of Mediterranean *Cavolinia inflexa* (Comeau et al. 2010b) and the Antarctic pteropod *Clio pyramidata* (Orr et al. 2005) have also been reported. The Arctic pteropod *L. helicina* is particularly vulnerable to the threat of ocean acidification because the Arctic Ocean is expected to be among the first regions that will exhibit large areas corrosive to calcium carbonate. Models project that undersaturation with respect to aragonite, the crystal form of $CaCO_3$ used by pteropods to build their shell, is going to occur as early as 2016 for 1 mo per year in 10% of the Arctic surface waters (Steinacher et al. 2009). Furthermore, aragonite undersaturation has already been detected in the Canada Basin of the Arctic Ocean in 2008 (Yamamoto-Kawai et al. 2009).

Our study aims to investigate the impact of ocean acidification on the early life stage of *Limacina helicina* from the Canada Basin of the Arctic Ocean. The present experiment, performed as a part of the Catlin Arctic Survey, was conducted at the end of winter on organisms collected below the ice floe. These unique spatial and temporal locations were used to better assess the effect of ocean acidification on organisms already submitted to natural unfavorable conditions. Mortality and shell linear extension responses to 3 controlled pH conditions (total scale pH [pH_T] ~8.05, 7.90 and 7.75) were investigated.

MATERIALS AND METHODS

This study was carried out as part of the Catlin Arctic Survey, which is designed to provide insight into the rate, causes and potential worldwide impacts of the disappearance of the Arctic sea ice cover. Sampling and experiments were undertaken in April 2010 in the Canadian Arctic from the Catlin Arctic Survey Ice Base located at 78.72° N, 104.79° W in

Deer Bay. The ice base was situated on a region of flat, first year sea ice that was between 1.5 and 1.7 m thick. Pteropods were collected from an ice hole located 2 km west of the ice base where the bottom depth was about 350 m. Sampling was performed using a plankton net (modified WP2 net with a 57 cm mouth diameter, 200 µm mesh size and a custom-made 10 l collector) that was gently trawled vertically from 200 m to the surface. Additional tows were performed from 100 m to the surface but only few organisms were collected. After collection, the organisms were maintained in iceboxes to avoid freezing and transported to the ice base. The organisms were selected under a microscope, and the few individuals exhibiting shell damage were eliminated. The nontoxic fluorochrome calcein was used as a growth marker to monitor the linear extension of the shell (e.g. Kaehler & McQuaid 1999). Before incubation, the shells of 198 pteropods were stained in a calcein fluorochrome bath for 1 h (final concentration: 50 mg l^{-1}) and were then rinsed by successive transfers in unstained seawater to remove the calcein that was not incorporated in the shell. The organisms were then divided among nine 125 ml incubation bottles (~22 ind. per bottle) containing seawater (collected at 5 m depth) adjusted to one of 3 pH values through additions of 0.1 N HCl (Merck). We are aware that gas bubbling and the addition of acid and bicarbonate and/or carbonate are the methods that best mimic anthropogenic ocean acidification, although acid addition generates changes in the pH, partial pressure of CO_2 (pCO_2) and $CaCO_3$ saturation state in the same direction as the changes generated by gas bubbling (Gattuso et al. 2010). The dire logistic and technical constraints in the ice base and tent laboratory prevented the use of equipment such as a balance, CO_2 cylinders or gas mixing pumps. The target pH values included a control treatment (pH_T ~8.05, pCO_2 ~380 µatm), a low pH treatment corresponding to the pCO_2 expected by 2050 (pH_T ~7.90; pCO_2 ~550 µatm) and a very low pH treatment corresponding to the pCO_2 expected by the end of the century (pH_T ~7.75; pCO_2 ~760 µatm). Seawater pH was measured in the experimental bottles at the beginning and at the end of the incubation using a pH meter (826 pH mobile, Metrohm) fitted with a glass electrode (Metrohm, electrode plus) calibrated on the total scale using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 33.0 (Dickson et al. 2007). Seawater samples for total alkalinity (A_T) determination were taken before and after incubation and poisoned with $HgCl_2$, and A_T was measured at the Laboratoire d'Océanographie de

Villefranche using potentiometric titrations with a Metrohm titrator (titrando 80) according to Dickson et al. (2007). Field samples were also collected to measure A_T and total dissolved inorganic carbon (DIC_T). Samples were also poisoned with $HgCl_2$ and stored pending measurement at the Institute of Ocean Sciences (British Columbia, Canada). Parameters of the carbon chemistry were determined from pH_T, A_T and/or DIC_T, temperature and salinity using the R package seacarb (Lavigne & Gattuso 2011). The incubation bottles were enriched with 5 ml (equivalent to 1 m^3 of the water column) of a solution of phytoplankton and particles collected in the field using a 20 µm plankton net lifted from a depth of 100 m to the surface. The bottles were incubated in seawater through an ice hole to maintain the temperature at the ambient level (ca. −1.6°C). Upon completion of the incubation (8 d), the active individuals were separated from the dead ones, which were discarded. Half of the active individuals were stored frozen but were damaged during the process and were not used for shell linear extension measurements. The other half was stored in a buffered formalin solution for subsequent measurement of linear extension. Photographs of the organisms were made at the Laboratoire d'Océanographie de Villefranche under UV-epifluorescence using a Zeiss Axiovert 25 microscope. The shell extension and shell diameter were measured on photographs using the software ImageJ (US National Institutes of Health). Shell extension corresponds to the linear growth of the shell during the 8 d following calcein staining (for details, see Comeau et al. 2009). To take into account differences in individual sizes, the ratio between shell extension and shell diameter was calculated.

Because the data were not normally distributed, a non-parametric Wilcoxon test was used to compare the 3 treatments by pairs.

RESULTS

The aragonite saturation state (Ω_a) in the field was particularly low, with a maximum Ω_a of 1.4 at 10 m (Table 1). Ω_a tends to decrease with increasing depth until 100 m, where it exhibits a minimum of 1.07. Ω_a was ~1.21 at 200 m, the maximum depth of pteropod collection. In the incubation bottles, Ω_a was also particularly low: the mean Ω_a over the incubation period was ~1.13 in the control treatment. In the other treatments, seawater was undersaturated with respect to aragonite, with mean Ω_a values of 0.79 and 0.59 in the low and very low pH treatments respectively (Table 1).

The incubated organisms suffered mortalities of 6, 8 and 9% in the control, low and very low pH treatments respectively. The organisms were of similar sizes in the 3 treatments, with a mean (±SD) maximum diameter of the shell of 1773 ± 230 µm. After 8 d of incubation, the ratio of linear extension (LE) to shell diameter (D) was 0.34 ± 0.1, 0.21 ± 0.1 and 0.17 ± 0.1 µm (LE) µm^{-1} (D) in the control, low and very low pH treatments respectively (Fig. 1). Wilcoxon tests revealed a significant difference (p < 0.01) in linear extension between pteropods maintained in the control treatment and those maintained at low and very low pH treatments, whereas there was no significant difference in the LE:D ratio between organisms maintained at low and very low pH treatments (p = 0.078). The shells of the juveniles main-

Table 1. Parameters of the carbonate chemistry in the 3 experimental conditions (control, low pH, very low pH) and in the field at 5, 10, 100 and 200 m depth. Mean ± SD is shown for the parameters that were measured (n = 6 for pH, and n = 9 for total alkalinity [A_T] and total dissolved inorganic carbon [DIC_T]). Total pH (pH_T) is the average of the mean pH values measured in the incubation bottles at the start (t_0) and end (t_f) of the incubation; for the field measurements it is calculated from DIC_T, A_T, salinity and temperature. A_T is the average of the mean total alkalinity measured in the incubation bottles at the start (t_0) and end (t_f) of the incubation; for the field measurements it is the measured values. The concentration of DIC_T, partial pressure of CO_2 (pCO_2) and saturation state of aragonite (Ω_a) were derived from pH_T, A_T, salinity and temperature in the experimental bottles and from DIC_T, A_T, salinity and temperature for field measurements

| Conditions | pH_T | | A_T (µmol kg^{-1}) | | pH_T | A_T (µmol kg^{-1}) | DIC_T (µmol kg^{-1}) | pCO_2 (µatm) | HCO_3^- (µmol kg^{-1}) | CO_3^{2-} (µmol kg^{-1}) | Ω_a |
	t_0	t_f	t_0	t_f							
Control	8.07	8.03	2143	2132	8.05 ± 0.03	2137 ± 8	2048	362	1949	74	1.13
Low pH	7.92	7.86	2102	2106	7.89 ± 0.04	2104 ± 3	2060	530	1971	52	0.79
Very low pH	7.78	7.74	2052	2065	7.76 ± 0.03	2058 ± 11	2046	704	1959	39	0.59
Field 5 m	–	–	–	–	8.14	2143 ± 7	2024 ± 5	287	1913	91	1.39
Field 10 m	–	–	–	–	8.14	2144 ± 5	2023 ± 2	285	1912	92	1.40
Field 100 m	–	–	–	–	7.96	2264 ± 2	2185 ± 3	459	2083	72	1.07
Field 200 m	–	–	–	–	7.99	2301 ± 2	2202 ± 4	430	2092	83	1.21
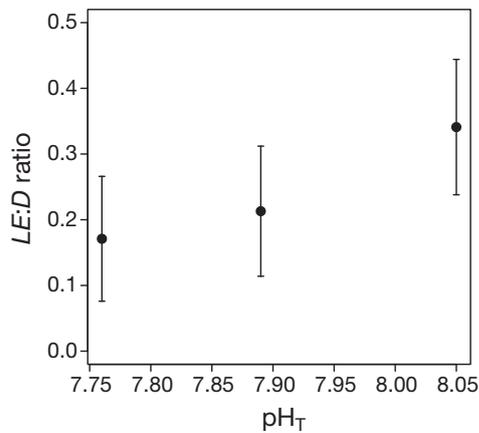
Fig. 1. *Limacina helicina.* Mean ±SD ratio of the shell linear extension (*LE*) and shell diameter (*D*) of juveniles after 8 d of incubation in control (total pH [pH_T] ~8.05), low pH (pH_T ~7.89) and very low pH (pH_T ~7.76) treatments. The ratio was significantly different ($p < 0.01$) between control and both low pH and very low pH treatments. There was no significant difference between the low and very low pH treatments ($p = 0.078$)

tained at the control pH were not altered during the incubation (Fig. 2a). In contrast, dissolution marks were visible on the shells of the organisms maintained at low pH (Fig. 2b), and large dissolution marks as well as cracks were present on the shell of pteropods maintained at very low pH (Fig. 2c).

DISCUSSION

The individuals of *Limacina helicina* investigated were collected in waters with a low Ω_a. Most of them were sampled at depths ranging between 100 and 200 m, where the saturation state is close to the threshold between supersaturation and undersatura-

tion with respect to aragonite (Ω_a ~1.0). The low saturation state of the Canada Basin has already been observed in 2008 by Yamamoto-Kawai et al. (2009). Those authors suggested that characteristics of the water masses, such as low salinity due to sea ice melt water and high organic matter re-mineralization in the layer between 100 and 200 m, explain the low saturation of these waters. This is in agreement with the higher concentrations of DIC_T that we measured at 100 and 200 m depth (DIC_T ~2200 µmol kg^{-1}) than at 5 and 10 m (DIC_T ~2020 µmol kg^{-1}).

To date, published studies on the impact of ocean acidification on Arctic pteropods have only been carried out in Svalbard (Comeau et al. 2009, 2010a, Lischka et al. 2011), at a latitude similar to the one of our study site (~79°N), but in a region with higher saturation states. For example, Comeau et al. (2010a) reported that the Ω_a at the surface in a fjord west of Spitsbergen was ~2. Physico-chemical properties, such as the influence of Atlantic water (Cottier et al. 2005) with high total alkalinity and higher temperature in the Greenland Sea, explain the lower sensitivity of western Svalbard waters to ocean acidification.

To our knowledge, the present study is the first one conducted in early spring conditions using late overwintering organisms. Despite ~24 h of sunlight, the phytoplankton concentration was still very low under the ice floe. A reduced food supply beginning the previous summer might increase the sensitivity of pteropods to physical stress at this time of the year. However, the results demonstrate that, despite relatively harsh conditions, juveniles were able to extend their shell even in corrosive waters. Pteropods are accustomed to being exposed to a low aragonite saturation state, particularly when they are overwintering in the deeper layer of the water column, and may, therefore, be able to calcify at a low Ω_a. The physiol-
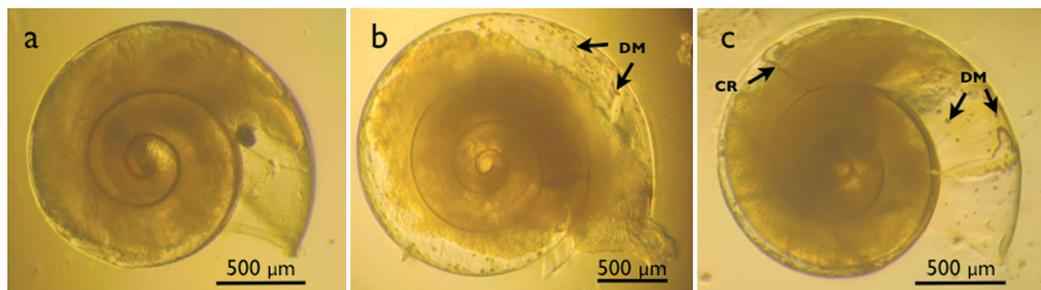

Fig. 2. *Limacina helicina.* Impact of seawater carbonate chemistry on the shell of juveniles maintained at 3 pH conditions for 8 d. Photographs were taken of organisms stored in a buffered formalin solution. Pteropods were able to extend their shell in the 3 pH conditions. Nevertheless, the shells of pteropods maintained at (a) control conditions (total pH [pH_T] ~8.05) were intact, whereas the shells exhibited dissolution marks (DM) at (b) low pH (pH_T ~7.89) and extensive DM and cracks (CR) at (c) very low pH (pH_T ~7.76)

ogy of calcification remains unknown in pteropods, but our data demonstrate that they are able to elevate Ω_a from seawater values as low as 0.6 to values >1 at the site of calcification. The presence of a thin periostracal layer covering the calcareous surface, as shown on the Antarctic pteropod *Limacina helicina antarctica* (Sato-Okoshi et al. 2010), might, among other mechanisms, protect the shell from a corrosive environment. Enzymatic reactions, such as the one involving carbonic anhydrase (e.g. Freeman & Wilbur 1948), an enzyme that catalyses the reversible hydration of CO_2 to HCO_3^- and H^+, might also be involved in the ability of pteropods to calcify in undersaturated waters. However, its presence and activity have not been demonstrated in pteropods to date.

Lischka et al. (2011) have also shown that larvae of *Limacina helicina* collected in Svalbard exhibited a positive linear extension of their shell after incubation in seawater undersaturated with respect to aragonite. Positive gross calcification in slightly undersaturated waters, measured using ^{45}Ca uptake, has also been reported on adults of *L. helicina* collected in Svalbard (Comeau et al. 2009, 2010a).

In the present study, only the linear extension of the shell was investigated. It must be pointed out that linear extension does not provide any information on the calcification rate because extension also depends on the density or thickness of the newly formed shell. The alkalinity anomaly technique was used to estimate the rate of net calcification, but the changes in total alkalinity were below the detection limit. Hence, even though our results demonstrate that pteropods are able to produce shell in corrosive waters, the results do not provide information on the balance between the precipitation and dissolution of $CaCO_3$. In corrosive waters, the whole pteropod shell is likely affected by dissolution, whereas calcification mostly occurs at the edge of the shell aperture. This is confirmed by the extensive dissolution marks on organisms maintained in undersaturated waters (Fig. 2b,c). Dissolution of the shell has also been observed in the Antarctic pteropod *Clio pyramidata* maintained in undersaturated waters with respect to aragonite (Orr et al. 2005) and in larvae of the Mediterranean species *Cavolinia inflexa* grown in seawater corrosive to aragonite (Comeau et al. 2010b). Model projections suggest that undersaturation of surface water with respect to aragonite will occur as early as 2016 for 1 mo per year in 10% of the Arctic Ocean (Steinacher et al. 2009). Subsurface waters are also affected by ocean acidification as models project that the aragonite saturation horizon

(the limit between undersaturation and supersaturation) will rise towards the surface substantially throughout the oceans (Orr et al. 2005). It is uncertain whether or how long pteropods will be able to maintain a positive balance between precipitation and dissolution of calcium carbonate.

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