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Invited feature

Limacina retroversa's response to combined effects of ocean acidification and sea water freshening

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ABSTRACT

Anthropogenic carbon dioxide emissions induce ocean acidification, thereby reducing carbonate ion concentration, which may affect the ability of calcifying organisms to build shells. Pteropods, the main planktonic producers of aragonite in the worlds' oceans, may be particularly vulnerable to changes in sea water chemistry. The negative effects are expected to be most severe at high-latitudes, where natural carbonate ion concentrations are low. In this study we investigated the combined effects of ocean acidification and freshening on *Limacina retroversa*, the dominant pteropod in sub polar areas. Living *L. retroversa*, collected in Northern Norwegian Sea, were exposed to four different pH values ranging from the pre-industrial level to the forecasted end of century ocean acidification scenario. Since over the past half-century the Norwegian Sea has experienced a progressive freshening with time, each pH level was combined with a salinity gradient in two factorial, randomized experiments investigating shell degradation, swimming behavior and survival. In addition, to investigate shell degradation without any physiologic influence, one perturbation experiments using only shells of dead pteropods was performed.

Lower pH reduced shell mass whereas shell dissolution increased with pCO_2. Interestingly, shells of dead organisms had a higher degree of dissolution than shells of living individuals. Mortality of *Limacina retroversa* was strongly affected only when both pH and salinity reduced simultaneously. The combined effects of lower salinity and lower pH also affected negatively the ability of pteropods to swim upwards. Results suggest that the energy cost of maintaining ion balance and avoiding sinking (in low salinity scenario) combined with the extra energy cost necessary to counteract shell dissolution (in high pCO_2 scenario), exceed the available energy budget of this organism causing the pteropods to change swimming behavior and begin to collapse. Since *L. retroversa* play an important role in the transport of carbonates to the deep oceans these findings have significant implications for the mechanisms influencing the inorganic carbon cycle in the sub-polar area.

1. Introduction

Increasing anthropogenic carbon dioxide emissions induce ocean acidification, thereby reducing pH, carbonate ion concentration, and calcium carbonate saturation state (Caldeira and Wickett, 2003; Doney et al., 2009). These changes in the carbonate system may negatively affect the ability of marine calcifying organisms to build their shells (Orr et al., 2005).

The last IPCC report (Intergovernmental Panel on Climate Change, 2007) recognized the influence of changes in sea water chemistry on calcareous organisms. The negative effects are considered to be especially severe in high-latitudes, where cold temperatures are responsible for an increase in CO_2 solubility and sensitivity of acid-base dissociation coefficients (Fabry et al., 2009).

In addition, the recent substantial increase in melting ice has led to an increase of melt sea water and therefore to an increase of fresh water that mixes with surface waters. This fresh water indeed has a lower concentration of Total Alkalinity (TA) and Dissolved Inorganic Carbon (DIC) which contribute to carbonate undersaturation. According to models, aragonite (a metastable form of calcium carbonate that is about 50% more soluble in sea water than calcite, Mucci, 1983) undersaturation is expected to take place in high latitude regions already by the middle of the century (Steinacher et al., 2009). In this scenario, pteropods, which use aragonite to build their shells, may be particularly sensitive to changes in sea water chemistry.

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Pteropods can occur in high densities in polar as well as in sub-polar waters (Lalli and Gilmer, 1989; Cooney et al., 2001; Tsurumi et al., 2005). They are the main planktonic producers of aragonite in the worlds' oceans (Seibel et al., 2007) and play a significant role in contributing to carbonate and organic carbon flux and sequestration (Fortier et al., 1994; Collier et al., 2000; Falk-Petersen et al., 2001; Pakhomov et al., 2002; Accornero et al., 2003; Karnovsky et al., 2008; Manno et al., 2010). Pteropods are an important component in the trophic chain. They are a major dietary component for some zooplankton and higher predators, such as herring, salmon, whales and birds (Hunt et al., 2008). Therefore, a decline of pteropod populations would likely cause dramatic changes to pelagic ecosystems. The dominant pteropod in polar waters is *Limacina helicina* whereas in the sub polar areas, *Clio pyramidata* and *Limacina retroversa* are present in high abundance (Van der Spoel, 1967). Recently, some investigations addressing the effects of increases in pCO_2 on *L. helicina*, have shown its vulnerability (e.g. increase in mortality, decrease in calcification/dissolution) to changes in pCO_2 (Comeau et al., 2009, 2010b; Lischka et al., 2011; Bednaršek et al., 2012). To our knowledge, only one investigation has been performed on *C. pyramidata* from the Subarctic Pacific (Orr et al., 2005; Fabry et al., 2008), showing that when exposed to high level of pCO_2, shell manifested marked dissolution within 48 h along its leading edge. Unfortunately, since the authors did not perform a replicated and controlled experiment, the results are not conclusive must be treated as a single case observation.

Limacina retroversa is an important grazer in high-latitude food chains, and it has been suggested to make substantial contributions to the transfer of carbon from the short-lived organic carbon pool in the surface waters, to the sequestered biogenic carbon pool in the deep sea (Legendre and Le Fevre, 1992; Bernard and Froneman, 2008). *Limacina retroversa* is also an important component of the diet of carnivorous macrozooplankton, micronekton and top vertebrate predators (Pakhomov and Perissinotto, 1996; Froneman et al., 1998; Armstrong, 2005). In the northern hemisphere, *L. retroversa* production peaks in the summer, but eggs production also occurs during spring and autumn phytoplankton blooms (Lalli and Gilmer, 1989; Gannefors et al., 2005). In the Argentine Sea, this pteropod exhibits a life cycle of two generations per year (Dadon and de Cidre, 1992). Individuals born in spring mature early, reproducing before the end of summer, whereas those born in summer delay sexual maturity, reproducing the following spring (Dadon and de Cidre, 1992). In the Norwegian Sea during autumn, this pteropod dominates the zooplankton community (Bathmann et al., 1991) and represents the maximum contribution to the biogenic particle flux (Honjo et al., 1988; Meinecke and Wefer, 1990). Over the past half-century, Norwegian Sea experienced a progressive deepening as well as amplification of the freshening with time (Curry et al., 2003; Dickson et al., 2007). That freshening has been attributed to some combination of enhanced wind-driven exports of ice or fresh water from the Arctic, increased net precipitation rates, and elevated volumes of continental runoff from melting ice.

Despite the important role of *Limacina retroversa* as pelagic mollusks and for vertical fluxes in both sub-Antarctic and sub-Arctic waters (Boltovskoy, 1971; Dadon, 1990), little attention has been devoted to the impact of ocean acidification on this species, and even less to the combined effect of ocean acidification and sea water freshening.

The aim of this study was to investigate the combined effects of lower pH and salinity on the pteropod *Limacina retroversa* from the Norwegian Sea. To assess this synergistic impact, mortality, shell mass, shell dissolution and locomotory speed were investigated by incubating the organisms in manipulated sea water.

2. Methods

Pteropods (*Limacina retroversa*) were collected by WP2 net (90 μm mesh size) at 50 m depth on October 2010 on board R/V Hyas at 69°53′48.48 N, 18°45′10.83 E, in Kvalsundet (Northern Norwegian coast, Fig. 1). As soon as they were collected, pteropods were stored in 25 l plastic containers filled with ambient sea water until they were transported back (within 2 h) to the laboratory at Tromsø University.

2.1. Perturbation experiment design

Pteropods were sorted and cleaned with 0.2 μm filtered sea water before being carefully transferred with a pipette to 400 ml closed borosilicate jars without head space to limit CO_2 exchange with atmosphere. We placed 8 individuals in each jar. To reduce the natural growth variability and since the calcification may vary with age and size of the organism, we used only individuals with similar sizes (700 μm).

Note that at present there are no published studies on the relationship between size and stage of *Limacina retroversa* in the Norwegian fjords. In the sub-Antarctic (Dadon and de Cidre, 1992; Bernard and Froneman, 2008) investigated the relation between size and stage and considered adults individuals >500–600 μm.

To fill the jars with the manipulated sea water, we used a plastic tube (extend from the bottle of the mother tank to the bottom of the jars) to minimize exchange of CO_2 with air. The experiment was performed in a cold room and the jars were placed in thermostated water tanks. The temperature was kept at 7 °C (in situ water condition). Sea water cultures were kept at four different pH (8.2, 8.0, low pH 7.8, and extremely low pH 7.6) ranging from the pre-industrial level (280 ppm), present day (350 ppm) level and the forecasted Ocean acidification scenario (750 and 1000 ppm).

Each pH level was combined with 3 different salinity dilutions (100%, 80% and 70%) of in situ salinity (33.8). Salinity dilutions 80% and 70% correspond respectively to the lower salinity during the Great Salinity Anomaly (Dickson et al., 1988) and to the surface layer during the summer melting ice (Wassmann et al., 2000). We performed 3 replicates for each treatment for a total of 3 × (4 pCO_2 × 3 salinity levels) = 36 jars.

In addition, 12 extra jars were prepared (3 replicates for each pH level) at control salinity for exposure of only dead pteropods. Pteropods were killed by $HgCl_2$ prior to incubation in order to investigate the shell degradation without any physiologic influence.

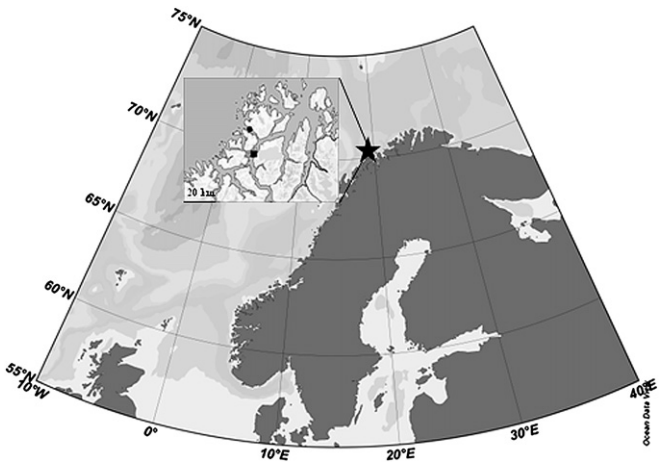

Fig. 1. Station where *Limacina retroversa* were collected on October 2010, Kvalsundet (Northern Norwegian coast) (black circle). Tromsø (black square).