Effect of elevated carbon dioxide concentrations on the growth of estuarine bivalve *Macoma balthica* from the Baltic Sea

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Ocean acidification

Since the pre-industrial times atmospheric CO_2 levels have increased over 100 ppm causing global warming (Widdicombe and Needham, 2007). Oceanic uptake of anthropogenic carbon dioxide is changing the carbonate chemistry of seawater, leading to lowering of pH. Ocean acidification has already reduced mean pH of seawater by 0.1 units (Range et al., 2011). Recent estimates suggest that continued release of CO_2 into the atmosphere will cause a further drop of pH by about 0.4 units by the end of the 21. century (Berge et al., 2006). A decrease in seawater pH may have serious consequences for marine biota at various levels of biological organisation (Bibby et al., 2008).
This study has been set up to investigate the impact of elevated CO_2 concentrations in water on growth of the Baltic clam *Macoma balthica*.

High CO_2 concentration experiment

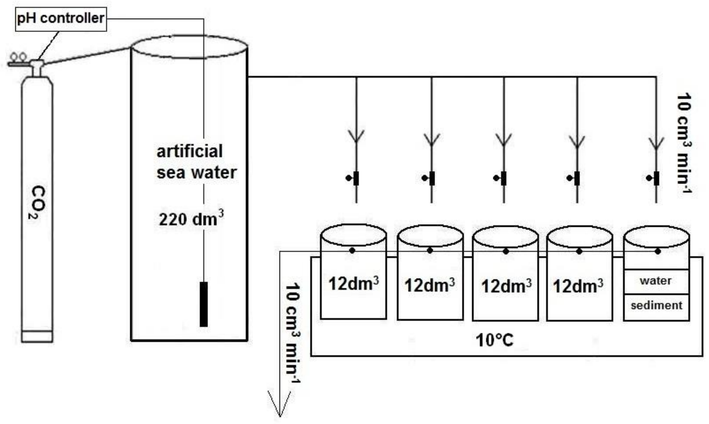

Fig.2 Scheme of Experimental system

Fig.1 The Baltic clam *Macoma balthica*

The impact of elevated CO_2 concentrations in water on growth of the Baltic clam *Macoma balthica* (Fig.1) was studied using four CO_2 levels: 400 ppm (control), 1000 ppm, 2000 ppm and 10000 ppm corresponding roughly to pH 7.7, 7.3, 7.0 and 6.3. One feeding regime was applied to all treatments under stable salinity (7.0) and temperature (10°C) conditions (Fig.2). Bivalves were collected in five replicates on the following days: start (0D) and then 1, 2, 4, 6 and 8 weeks (W) after introduction of CO_2.

Growth of shell length was determined in bivalves of similar size (11.4 - 14.99 mm) using fluorochrome marking (250 mg dm^{-3} calcein shell staining for 24 h before the exposure). Internal calcein mark deposited in shell was measured using 200 µm cross-cut sections incorporating the maximum growth axis (at the shell tip - blue line and at the shell side - red line; Fig.3) of the shell embedded in the epoxy resin (Fig.4). Shell increase was observed through fluorescence microscopy excitation from 460 to 490 nm (blue light). For shell length (blue line) relative growth (RG) was then calculated as a ratio of growth measurement and shell length multiplied by 1000.

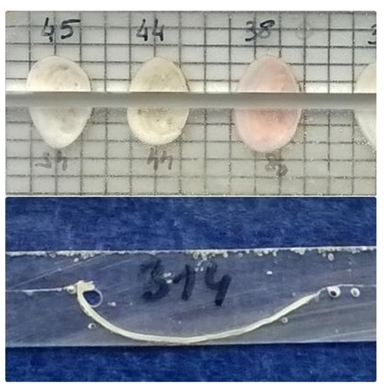

Fig.4 *M. balthica* shells embedded in epoxy resin

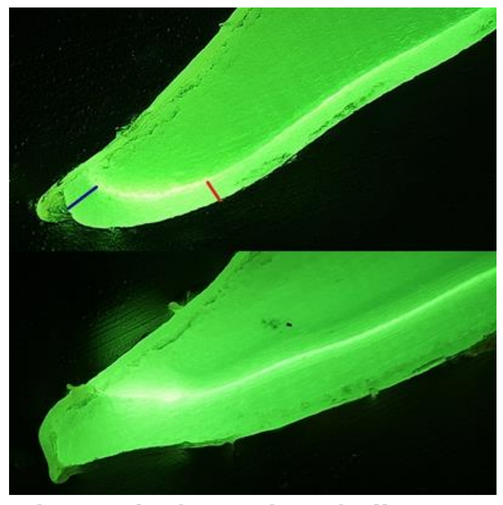

Fig.3 Calcein marks; shell growth in length and thickness.

Results and conclusions

Relative growth varied significantly between water pH (CO_2 concentrations; Kruskal-Wallis ANOVA $H_{3,198}$=9.305, p<0.05) and differed over time (Kruskal-Wallis ANOVA $H_{5,198}$=27.764, p<0.05; Fig.5).

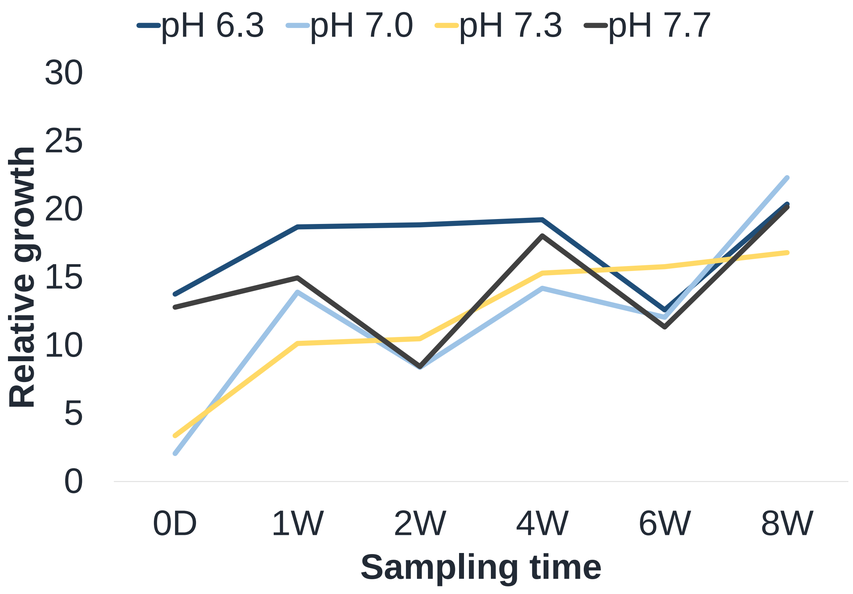

Fig.5 Relative growth of shells exposed to different water pH. Data are presented as means (n=5-13).

Differences in growth were also significant for shell thickness (Fig.6) among CO_2 concentrations (Kruskal-Wallis ANOVA $H_{3,294}$=11.232, p<0.05) and temporally (Kruskal-Wallis ANOVA $H_{5,294}$=44.362, p<0.05).

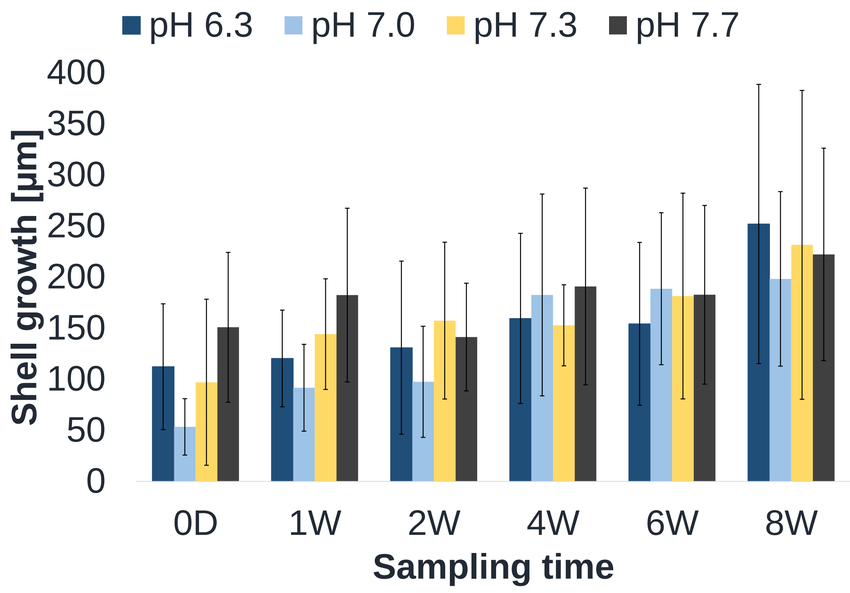

Fig.6 Shell growth in thickness exposed to different water pH. Data are presented as means±SD (n=5-13).

- Elevated CO_2 concentrations influence *M. balthica* shell growth in length and thickness.
- Water pH 6.3 causes slowest shell growth in thickness.
- Relative growth of shell is lower after 2 and 6 weeks of exposure to elevated CO_2 concentrations in water.

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