Behavioural and physiological responses of the estuarine bivalve *Macoma balthica* from the Baltic Sea to increased CO_2 concentration

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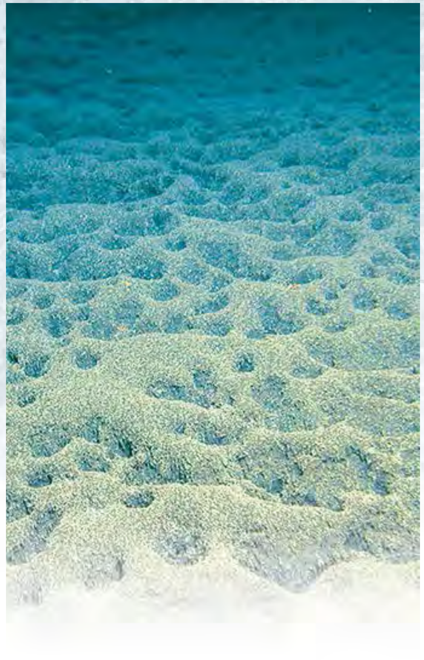
ECO$_2$

Sub-seabed CO$_2$ Storage:
Impact on Marine Ecosystems



EU RTD 2011-2015 (http://www.eco2-project.eu/)

In the atmosphere

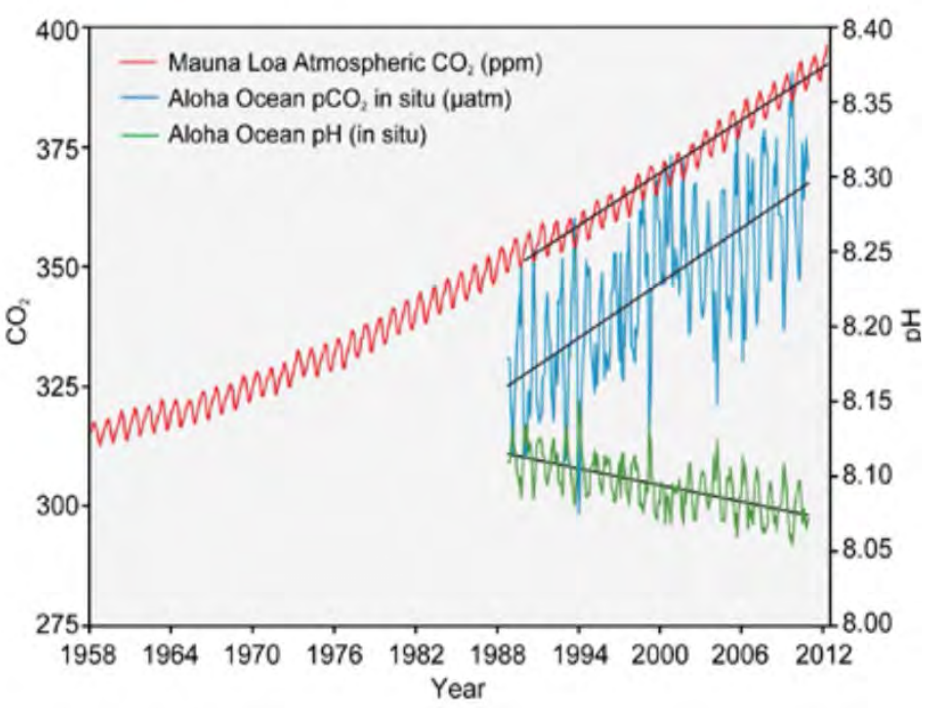
Carbon dioxide (CO_2) has continued to rise since the start of the industrial evolution (ca. 1750) but current concentrations are not particularily unusual in the Earth’s history

(http://www.esrl.noaa.gov)

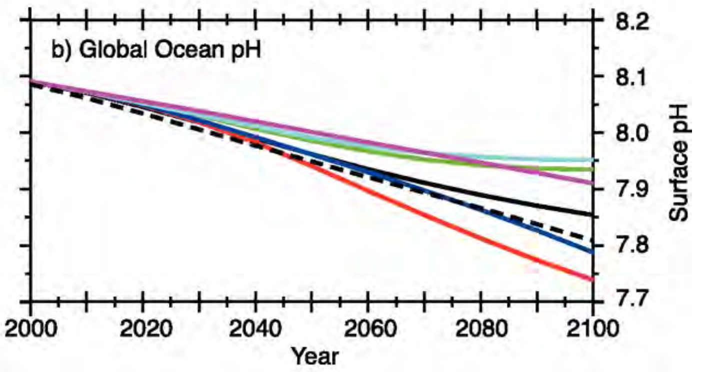
In the marine environment

50% of anthropogenic CO_2 has been taken up by the World Ocean over the last 250 yrs (Sabine et al., 2004) ⇨ decline in water pH

Predictions: seawater pH will fall by up to 0.4 units before 2100 and by 0.7 units by 2250



(http://nca2014.globalchange.gov)



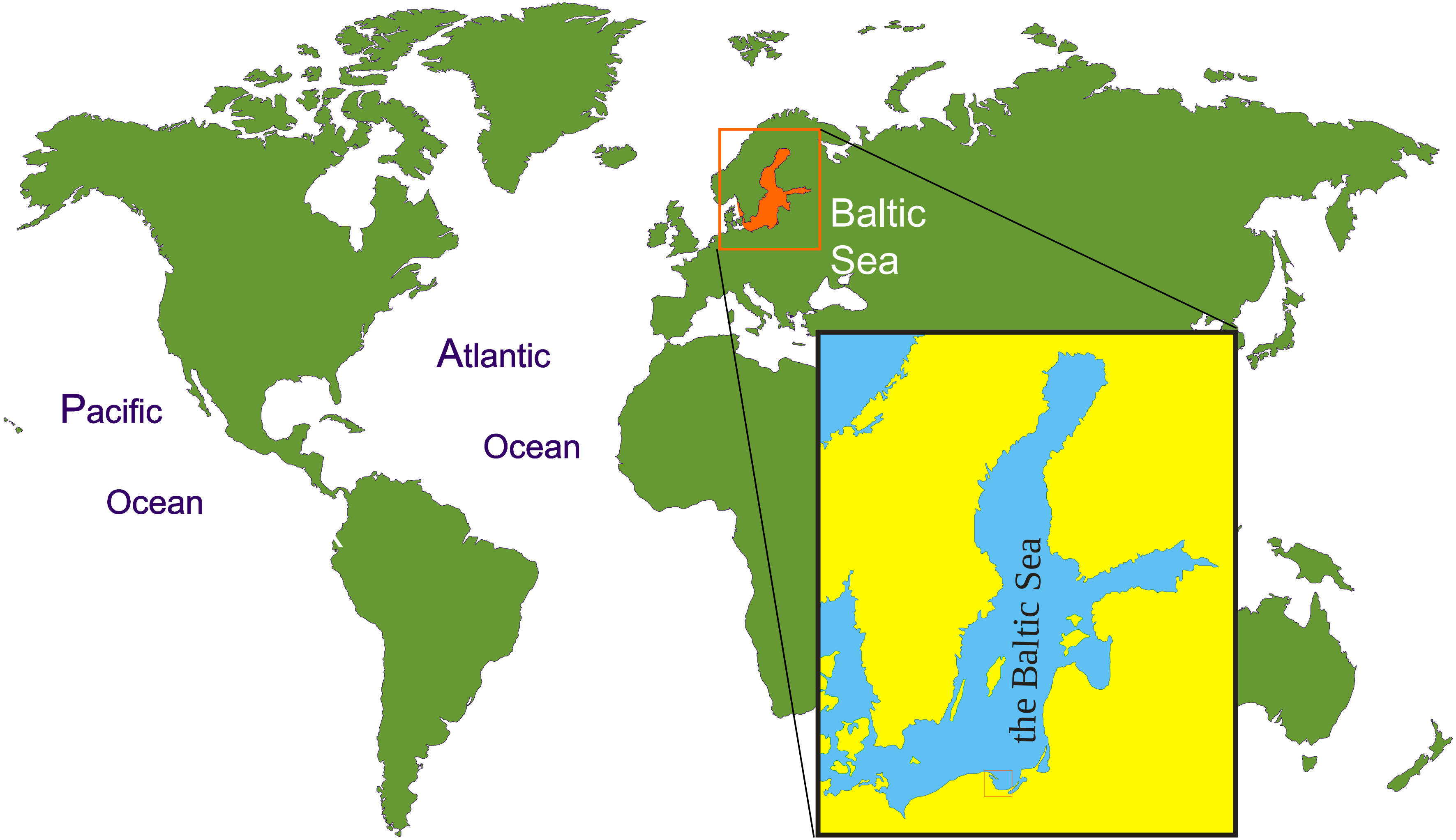
(http://www.ipcc.ch)

Managing ecological risks from increased seawater pCO_2 and a decline in pH remains an important challenge

The impact of high CO_2 concentration

can be particularly evident in water basins which are subject to other abiotic stressors, including eutrophication and anthropogenic pressure.

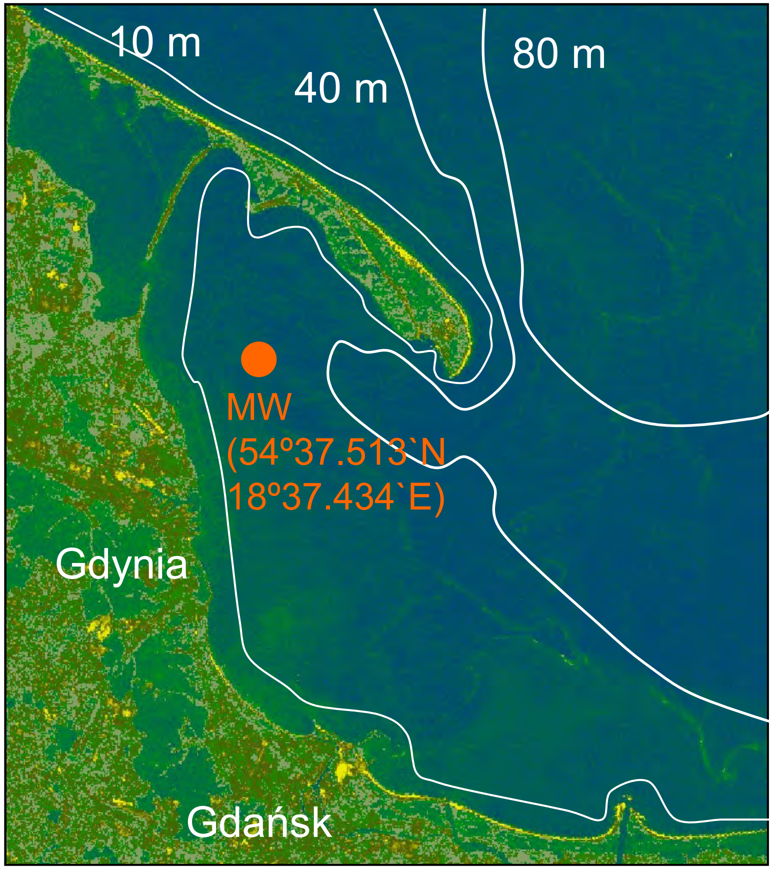
the Baltic Sea, coastal sea in central Europe



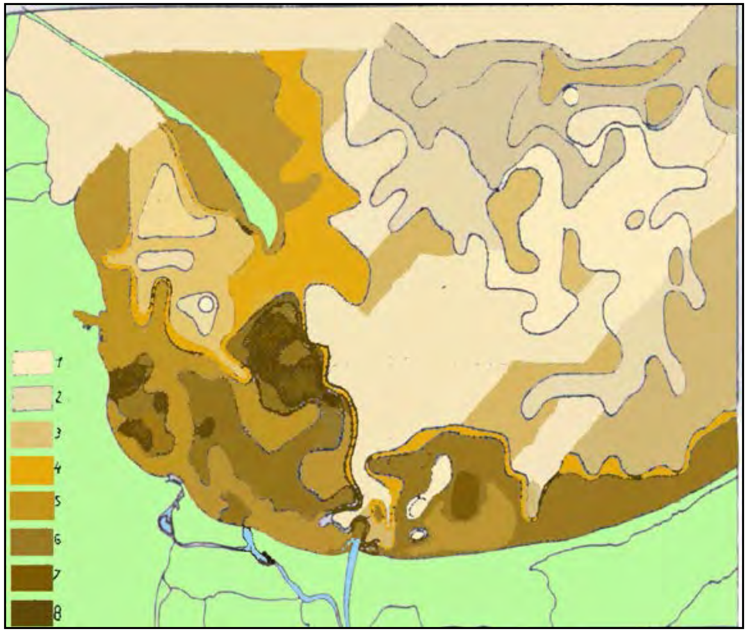
Sampling area

Main features of the Gulf of Gdańsk

- low salinity (brackish system) ⇨ osmotic stress
- eutrophication, high sediment organic matter content (short- and long-term hypoxia and anoxia)
- strong human pressure
- predicted decline in pH and in increase of hypoxic area (Omstedt et al., 2012)



Sediment type



Baltic clam *Macoma balthica* (L.)

- an infaunal tellinid bivalve with calcified skeleton
- occupies sandy and muddy bottoms (Hummel et al., 1997)
- commonly present in marine and estuarine habitats along both sides of the North Atlantic

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tolerant to adverse environmental conditions(e.g. low O_2 concentration and even short-term presence of H_2S)

at high metabolic costs

low ecophysiological performance
higher sensitivity to additional stress

→

POTENTIAL EFFECT OF INCREASED CO_2 AT POPULATION AND ORGANISM LEVELS

alterations in functioning, affecting various aspects of the clam's biology and ecology

Aim of study:

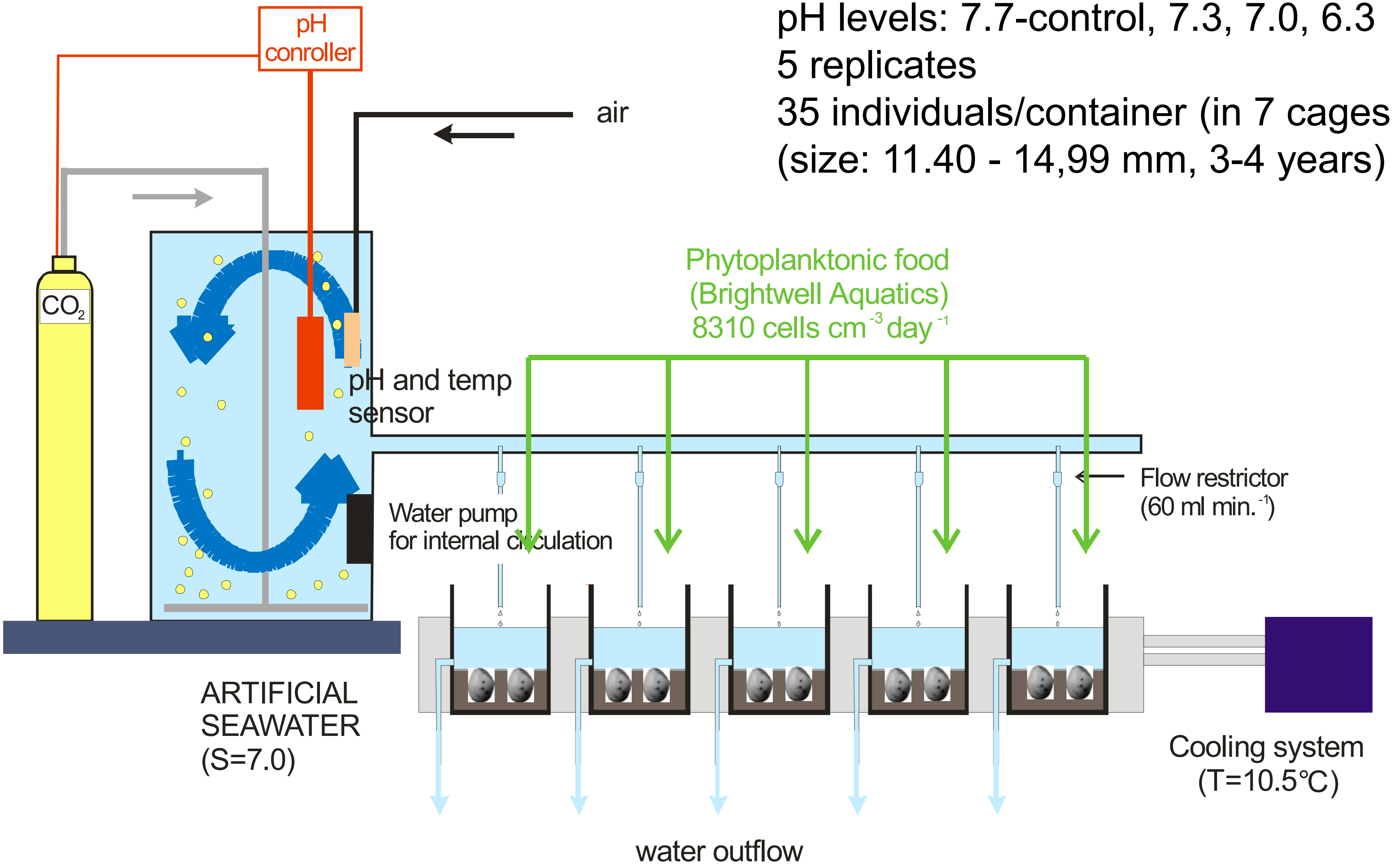
Behavioural and physiological responses of the estuarine bivalve *Macoma balthica* from the Baltic Sea to increased CO_2 concentration

The study was designed specifically:

- to assess the impact of reduced water pH on bivalves using a set of behavioural indicators (mortality, burrowing depth) and physiological markers (growth rate, content of biochemical compounds and respiration rate)

Experimental set-up

Open flow-through thermally-controlled system



pH levels: 7.7-control, 7.3, 7.0, 6.3
5 replicates
35 individuals/container (in 7 cages)
(size: 11.40 - 14,99 mm, 3-4 years)

Experimental set-up

Sampling schedule

3 months (11.04 – 6.07.2014):
- collection in the field,
- 31-day acclimatisation (no CO_2), 0d
- 7d, 2w, 4w, 6w, 8w

Biological responses

Behavioural:
- survival,
- burrowing depth (cm)

Physiological:
- morphological condition indices (CI)
- respiration rate (mlO_2 g DW^{-1} h^{-1})
- relative growth rate
- content of biochemical compounds (lipids, carbohydrates, glycogen, proteins; %)

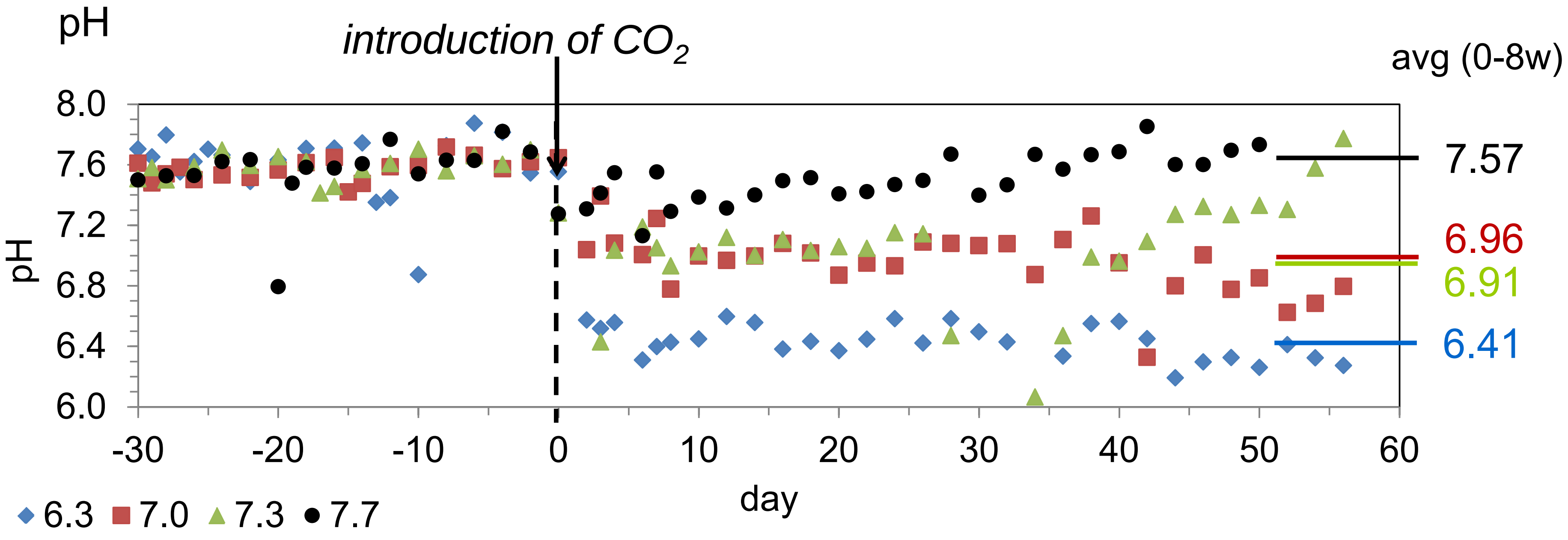
Water and sediment variables

Water: T, S, pH, O_2 – everyday
KH (carbonate hardness), total alkalinity (TA) – every 3rd day
Sediment pore water: pH, Eh (redox potential) – day of bivalve sampling

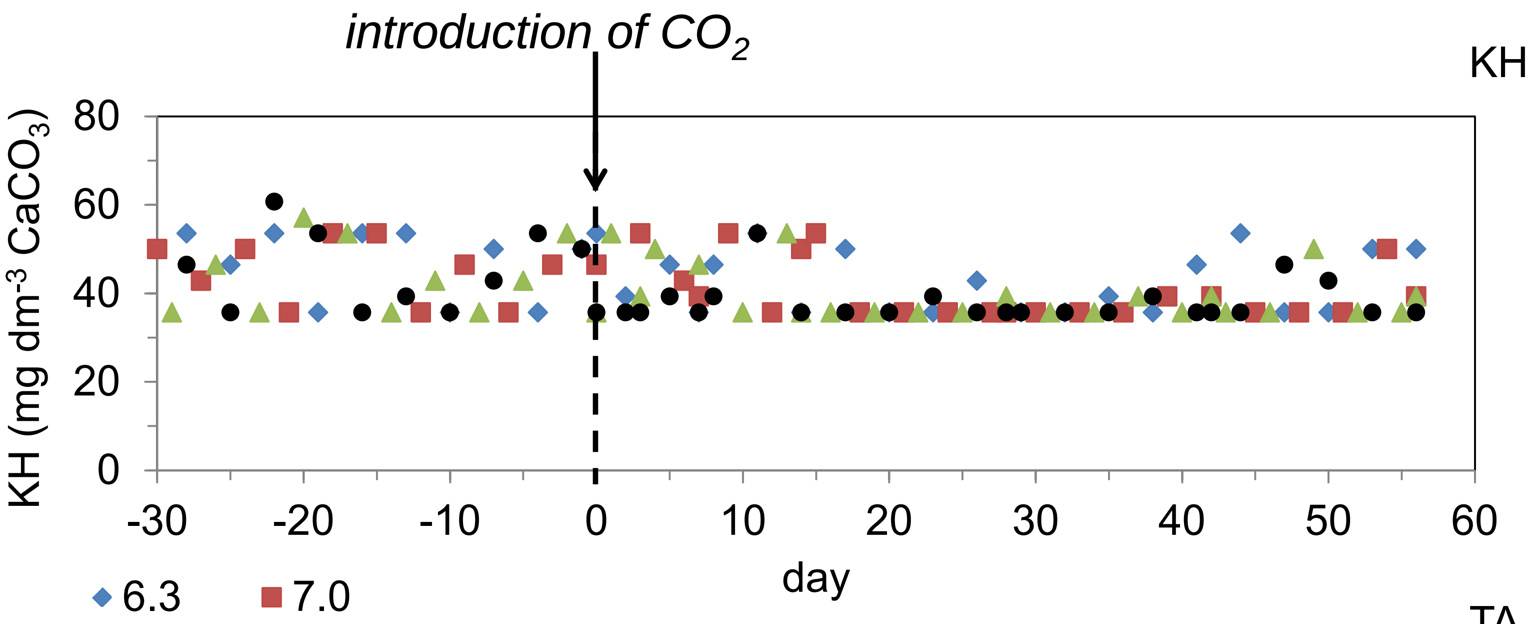
Experimental conditions

(mean±st.err., n=278-280)

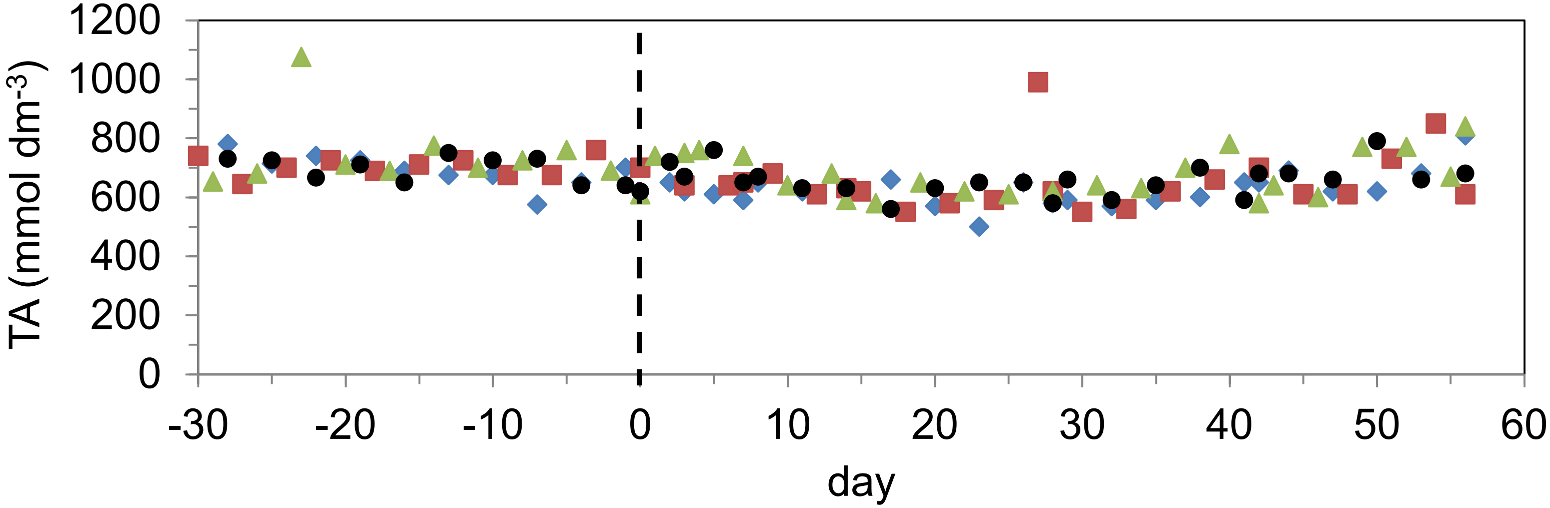
exp. pH	T (°C)	S	O_2 (mg dm^{-3})
7.7	10.70±0.10	6.96±0.00	4.95±0.06
7.3	10.66±0.09	6.95±0.01	5.11±0.05
7.0	10.64±0.09	6.92±0.01	5.18±0.07
6.3	10.56±0.09	6.93±0.01	5.33±0.08



Experimental conditions cont.

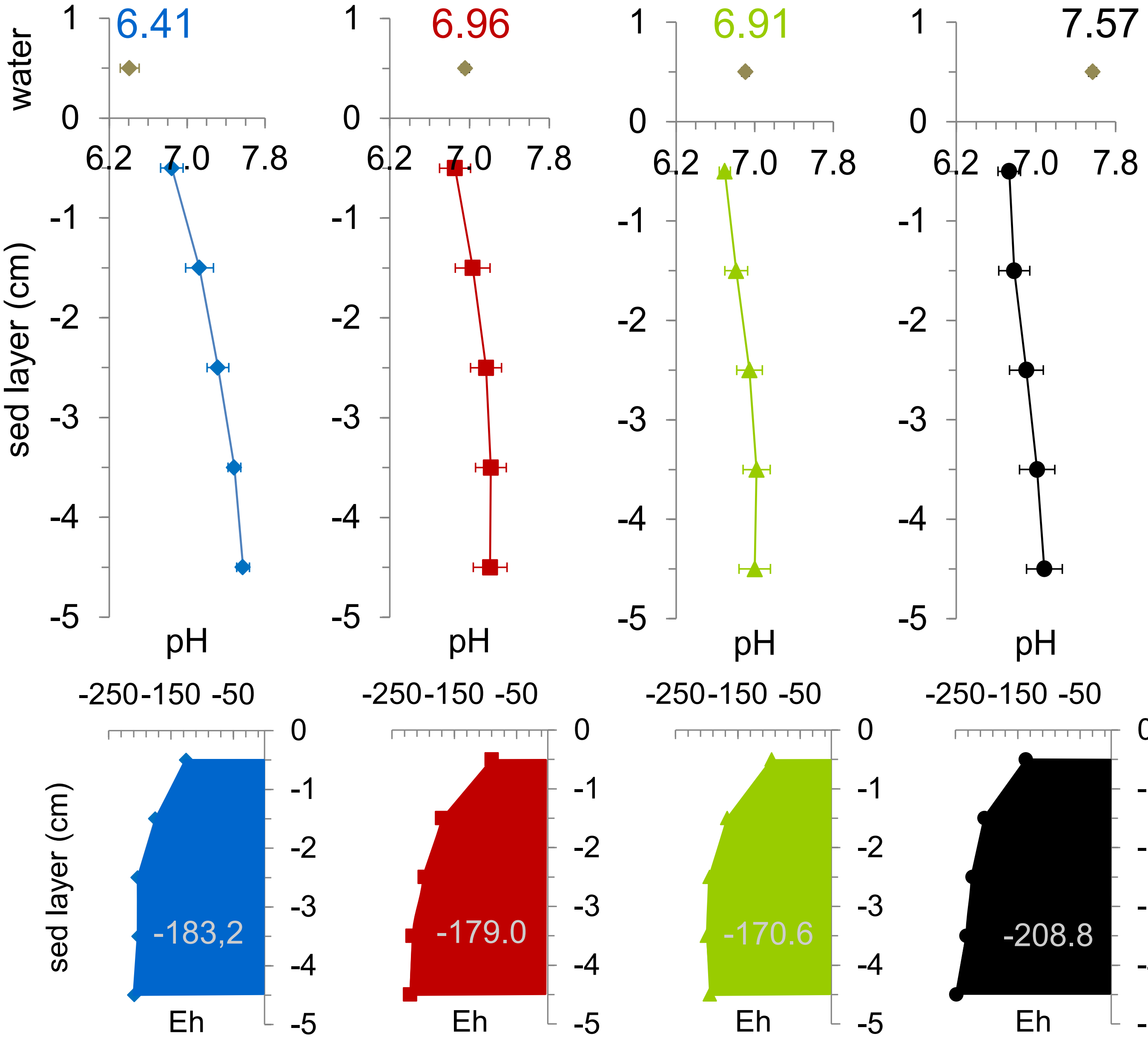


KH avg (0-56d)	
42.1	◆ 6.3
40.4	■ 7.0
39.7	▲ 7.3
37.9	● 7.7



TA avg (0-56d)	
654	◆ 6.3
668	■ 7.0
697	▲ 7.3
668	● 7.7

Experimental conditions in sediment pore water



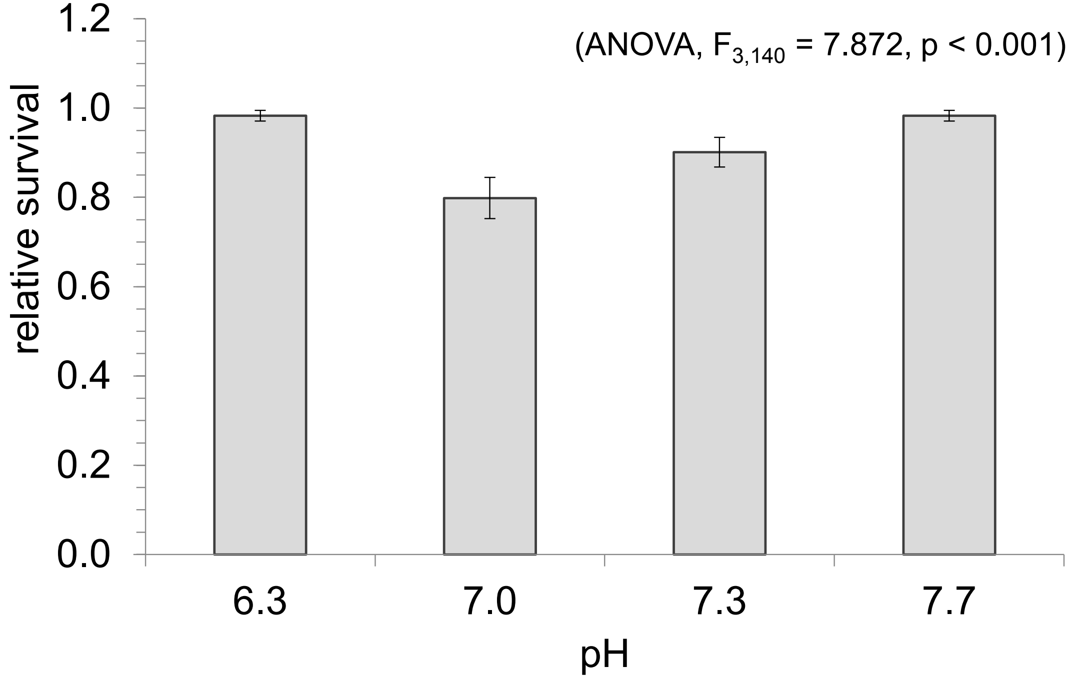
pH reducing effect,
SO_3^{-2} reduction,
presence of H_2S

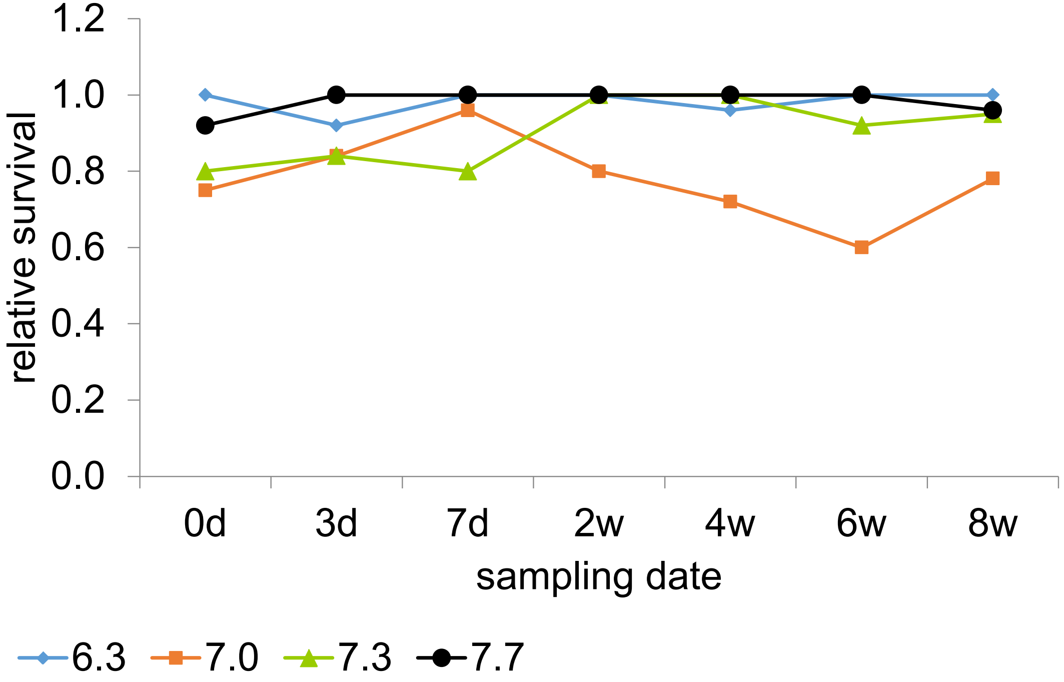
degradation of organic matter,
anoxic conditions

Biological responses

Relative survival
(mean±SE)

reduced survival
with water acidification
(exception pH=6.3),
tendency to decrease
survival with time





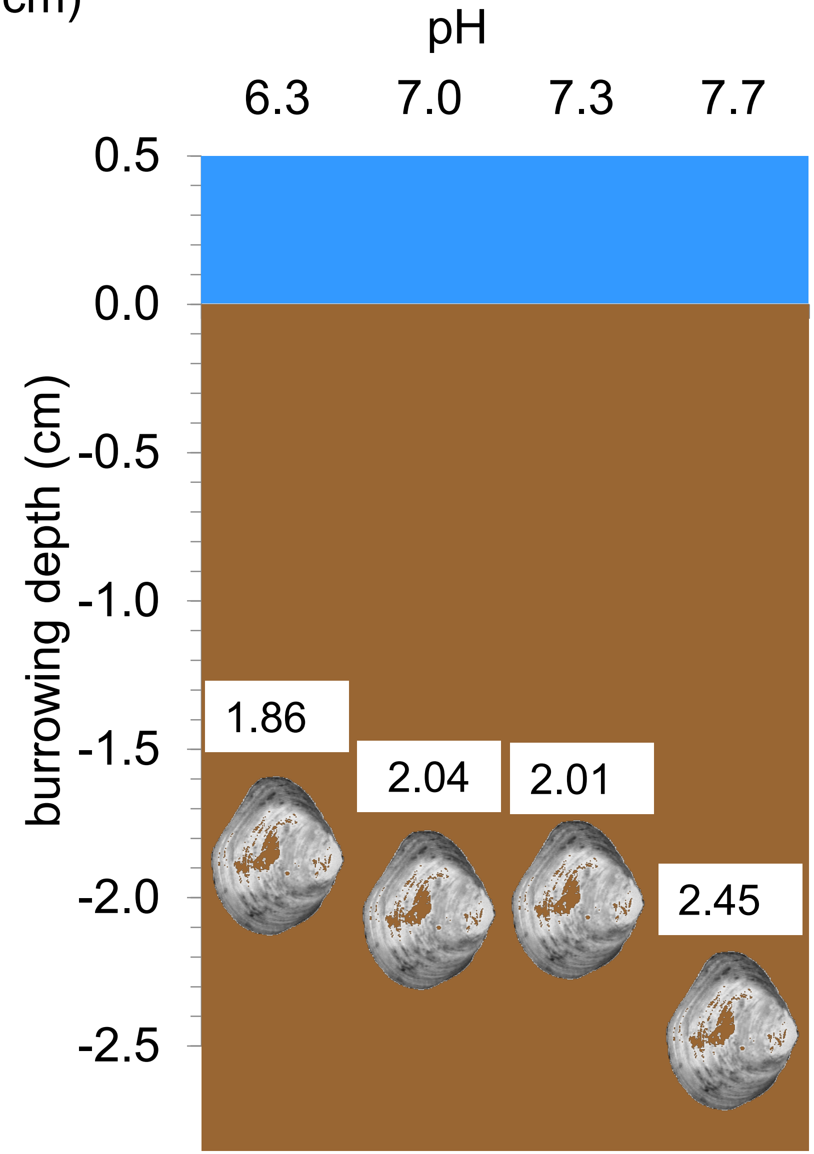
Biological responses

Burrowing depth of living individuals (cm)
(mean for all sampling dates, n=25)

decreased burrowing depth with decreasing water pH

behavioural stress-response to acidification

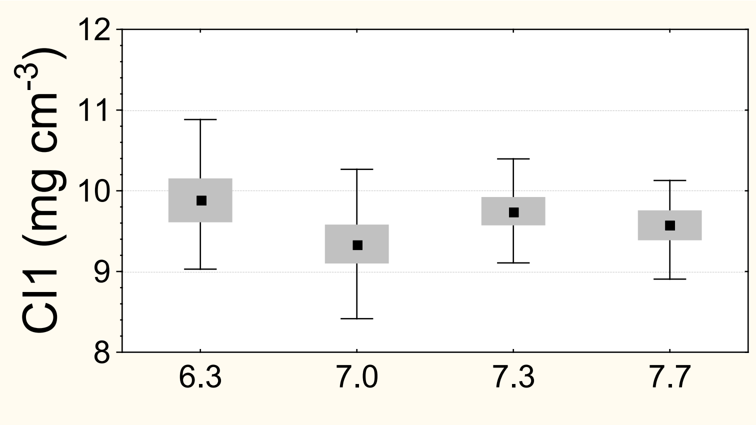
(ANOVA, $F_{3,447} = 2.736$, $p < 0.05$)



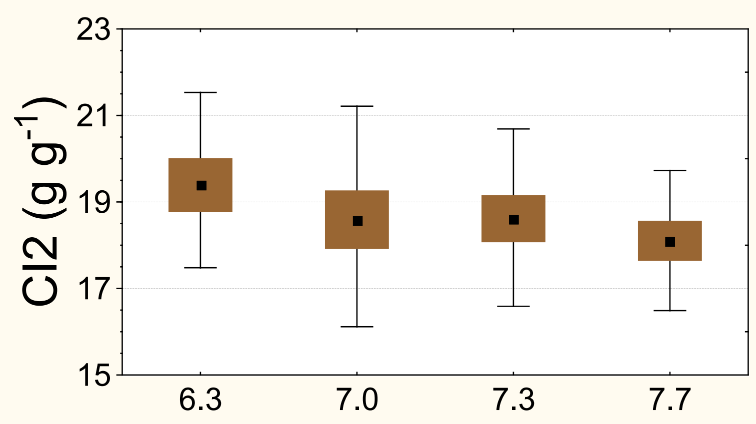
Biological responses

Condition indices (CI)

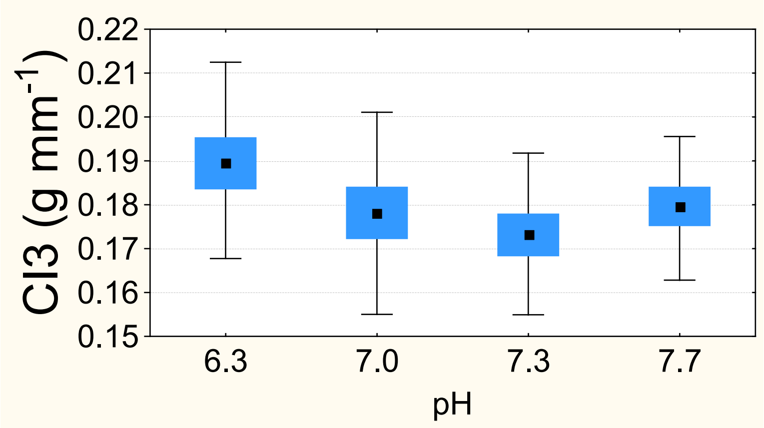
Morphological condition indicex 1 (**CI1**)
- soft tissue DW · shell length^{-3}
(mg cm^{-3})



Condition indicex 2 (**CI2**)
- soft tissue DW · shell dry weight^{-1}·100
(g g^{-1})

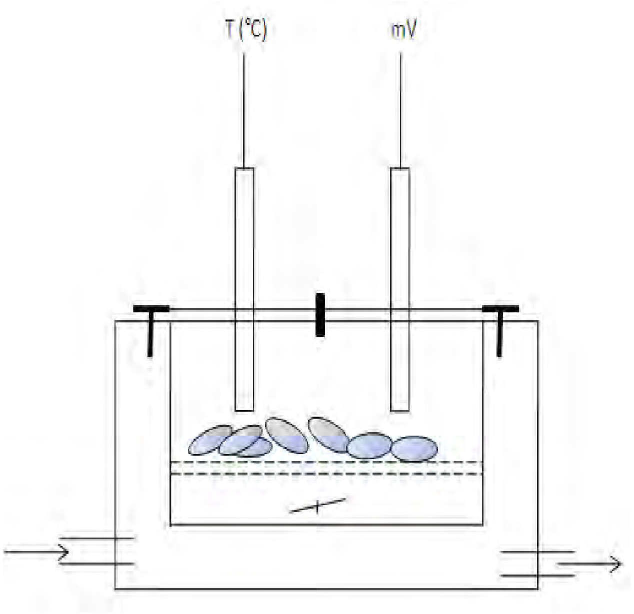


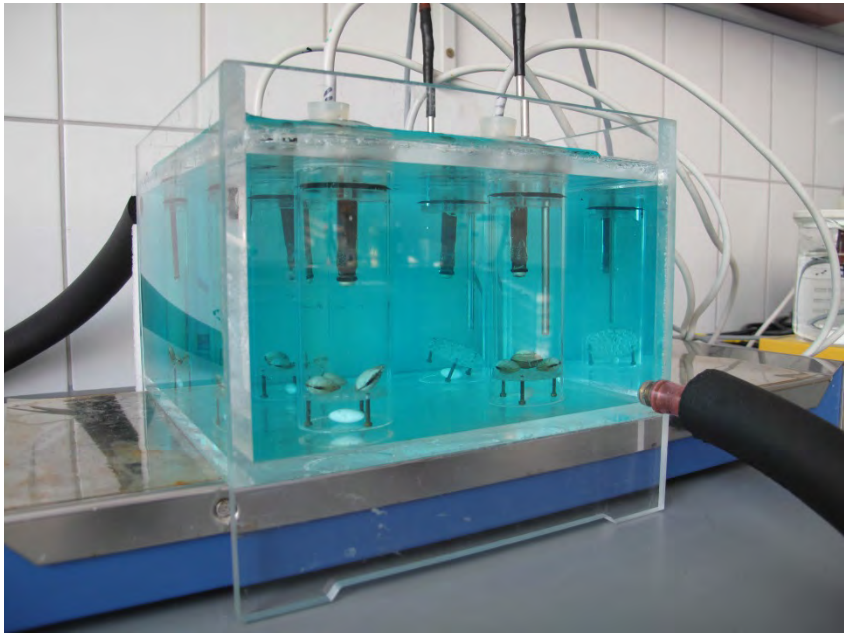
Condition indicex 3 (**CI3**)
- soft tissue DW · shell length^{-1}·100
(g mm^{-1})



Biological responses

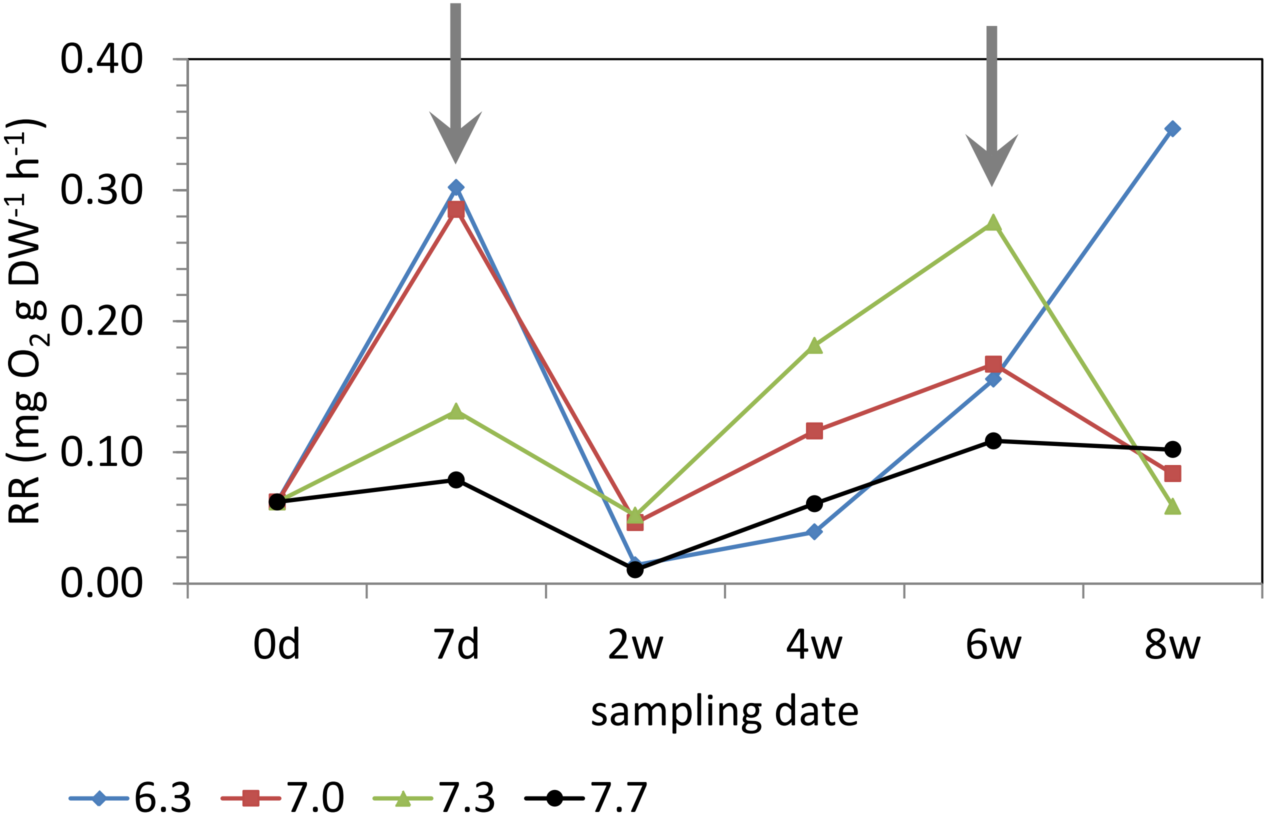
Respiration rate (RR; *proxy of metabolic rate*)
- animals purged over 24 h in Whatman GF-F filtered sea water
- clear behavioural activity (siphon and/or foot)
- 3 replicates, 3 individuals per chamber
- measurement of oxygen tension using Clark-type O_2 electrodes at 10°C in temperature-controlled respiration chambers over ca. 150 min.
- O_2 consumption (mlO_2 g DW^{-1} h^{-1})





Biological responses

Respiration rate, temporal acclimatisation

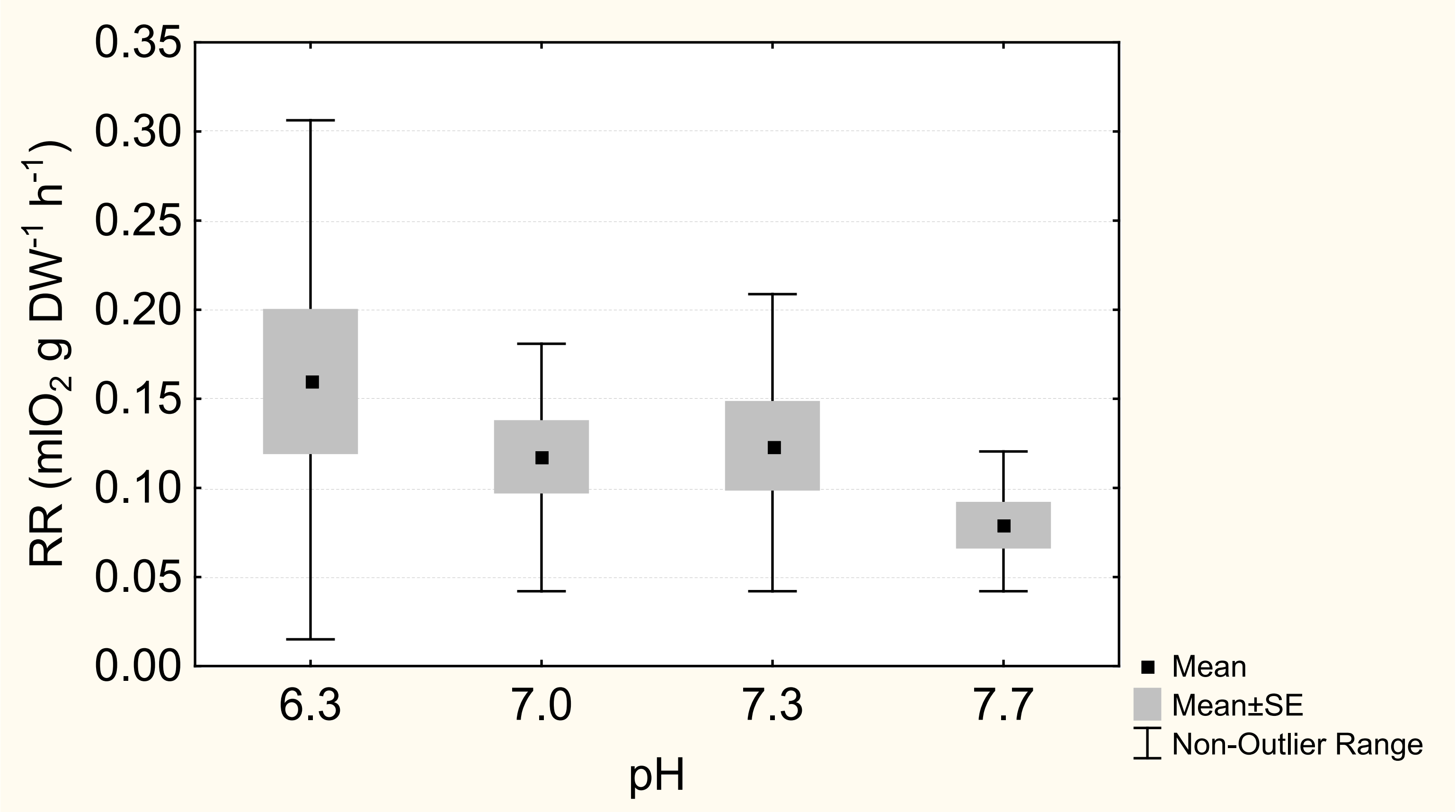


two-phasic acclimatization pattern with increased metabolic activity 7days and 6 weeks after introduction of CO_2
RR after 8 weeks similar to initial (except pH = 6.3)

Biological responses

Respiration rate

(ANOVA, $F_{3,60} = 1.227$, $p > 0.05$)

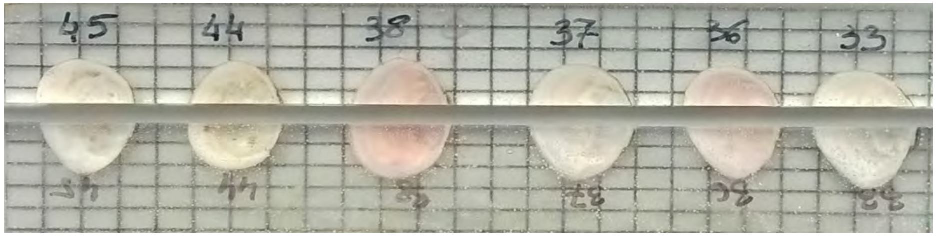


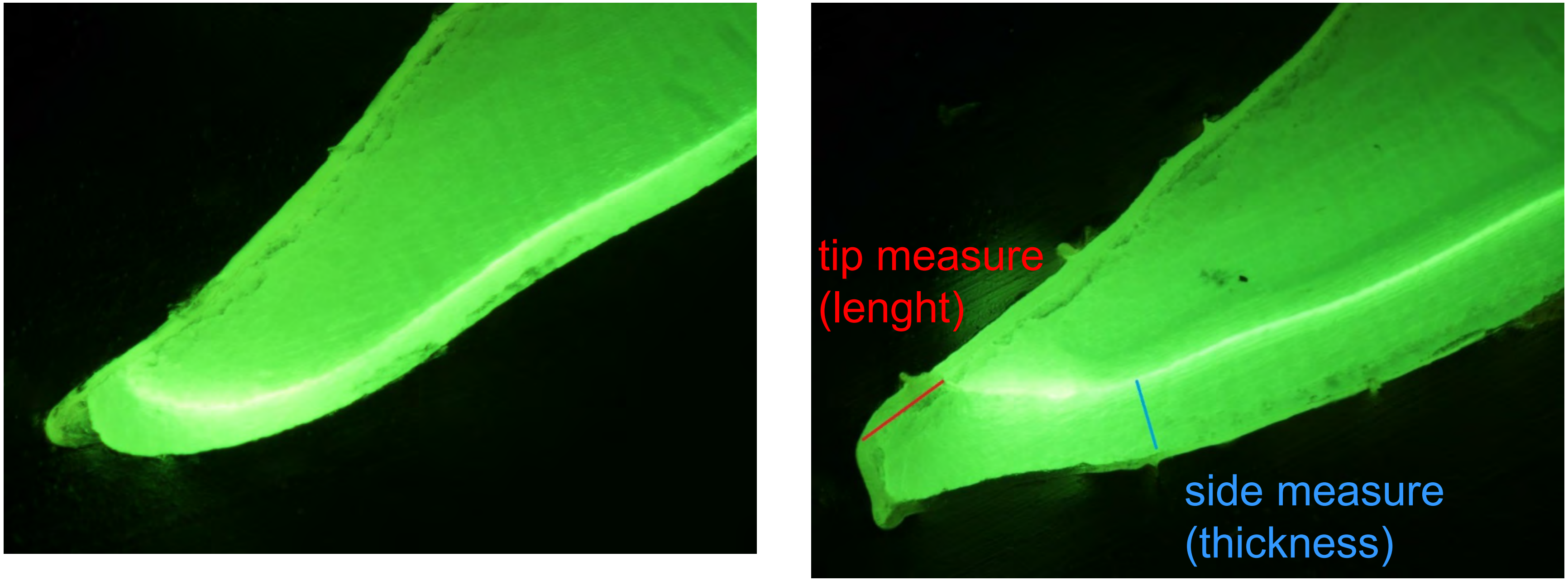
RR tends to increase at lower pH (high temporal variability); higher CO_2 concentrations induce higher metabolic rate to meet larger energy requirements. Less energy allocated to other living processes.

Biological responses

Relative growth rate

- 15 – 21 individuals per sample
- calcein staining for 24 h
- observation of shells embedded in epoxy raisin in epifluorescence at 460-490 nm (e.g. Mahé et al., 2010)





Correlation: tip *vs.* side ($R = 0.805$; $p < 0.001$)

Biological responses

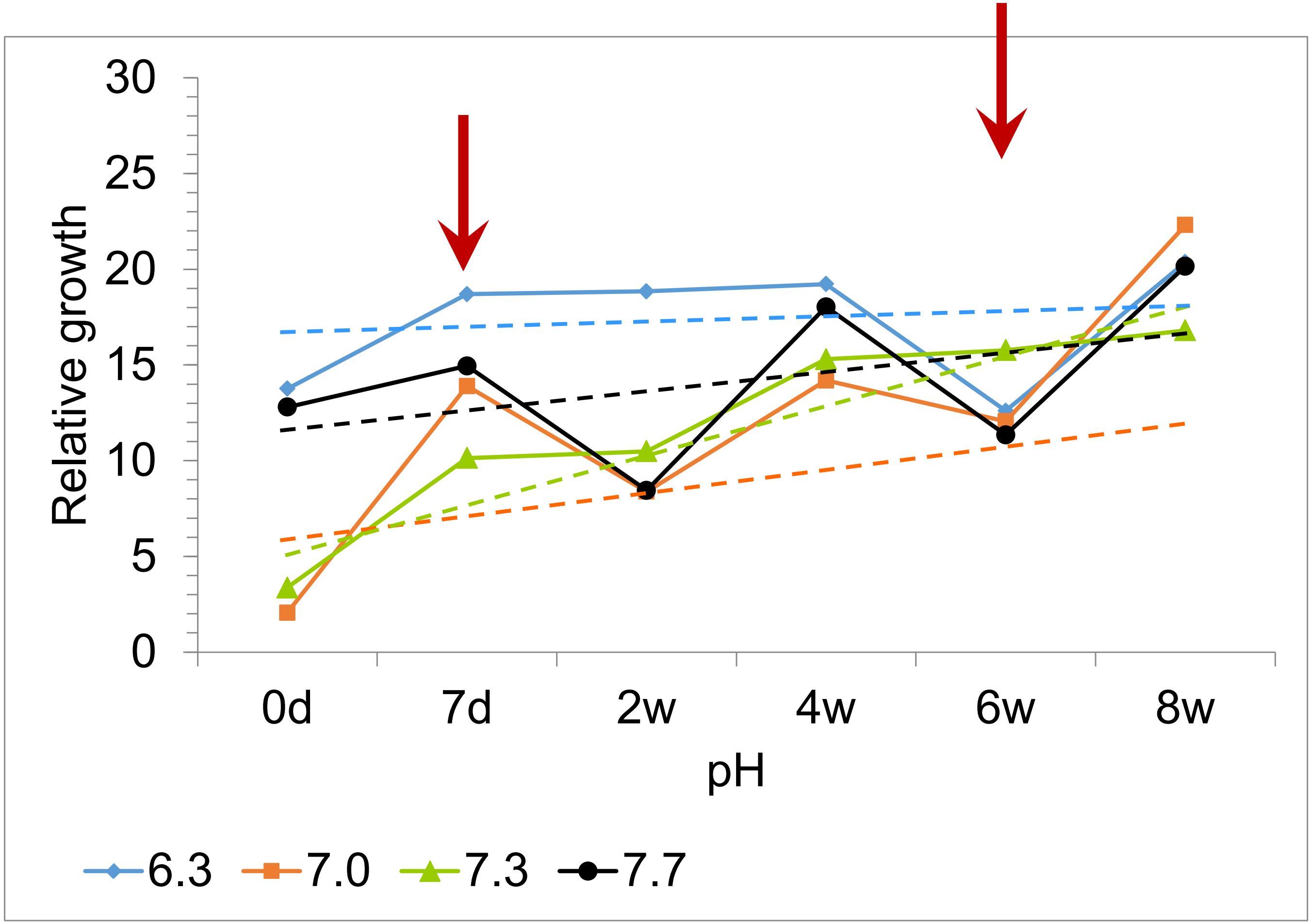
Relative growth rate (RG): tip measure shell length^{-1} * 1000 (‰)

n = 174

(Kruskal-Wallis ANOVA, $H_{3,198}$ = 27.467, p < 0.001)

Slope of regression line:

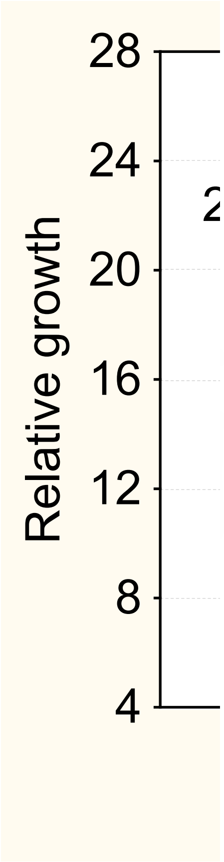
pH = 6.3	0.432
pH = 7.0	2.900
pH = 7.3	2,540
pH = 7.7	1.016

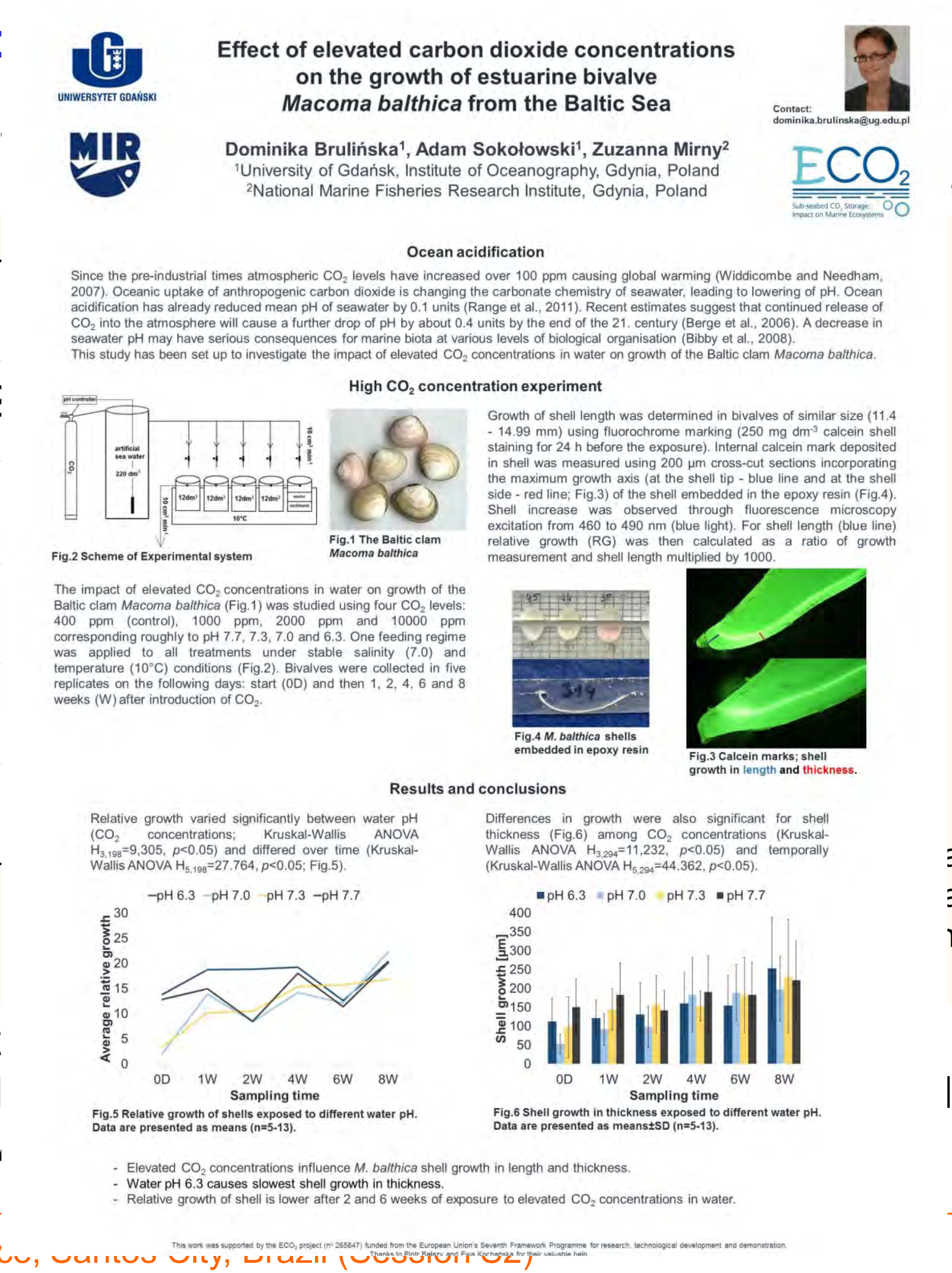


Biological respo

Relative growth r
(mean)

98 = 9.305, p < 0.03)





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

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ECO$_2$ Sub-seabed CO_2 Storage: Impact on Marine Ecosystems

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*

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.

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.

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).

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

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.

This work was supported by the ECO$_2$ project (no 265847) funded from the European Union's Seventh Framework Programme for research, technological development and demonstration.
Thanks to Piotr Balazy and Ewa Kochańska for their valuable help.

slower rat
enhanced
shell grow

located into

Biological responses

Ecophysiological role and metabolism of energetic reserves

SOURCE → METABOLIC TURNOVER → CATABOLISM AND USE

Glycogen

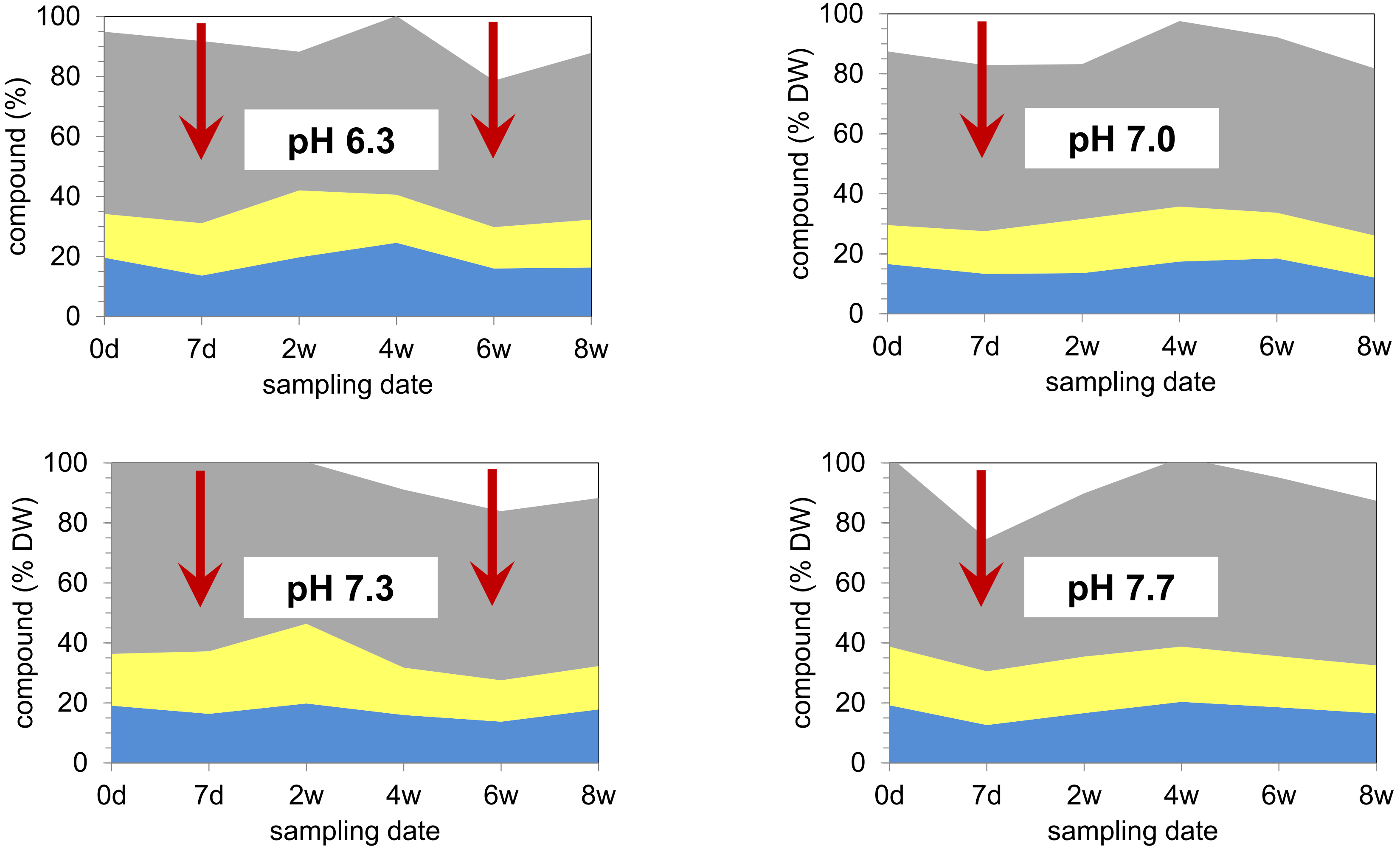
- food	- simple absorption (fast gain) - relatively fast turnover - intermediate energetic value	- needs of maintainance and functioning under stress (e.g. spawning, adverse conditions)

Lipids

- food - conversion from glycogen	- slow gain - slow metabolic turnover - high energetic value	- energy source for „long” stress

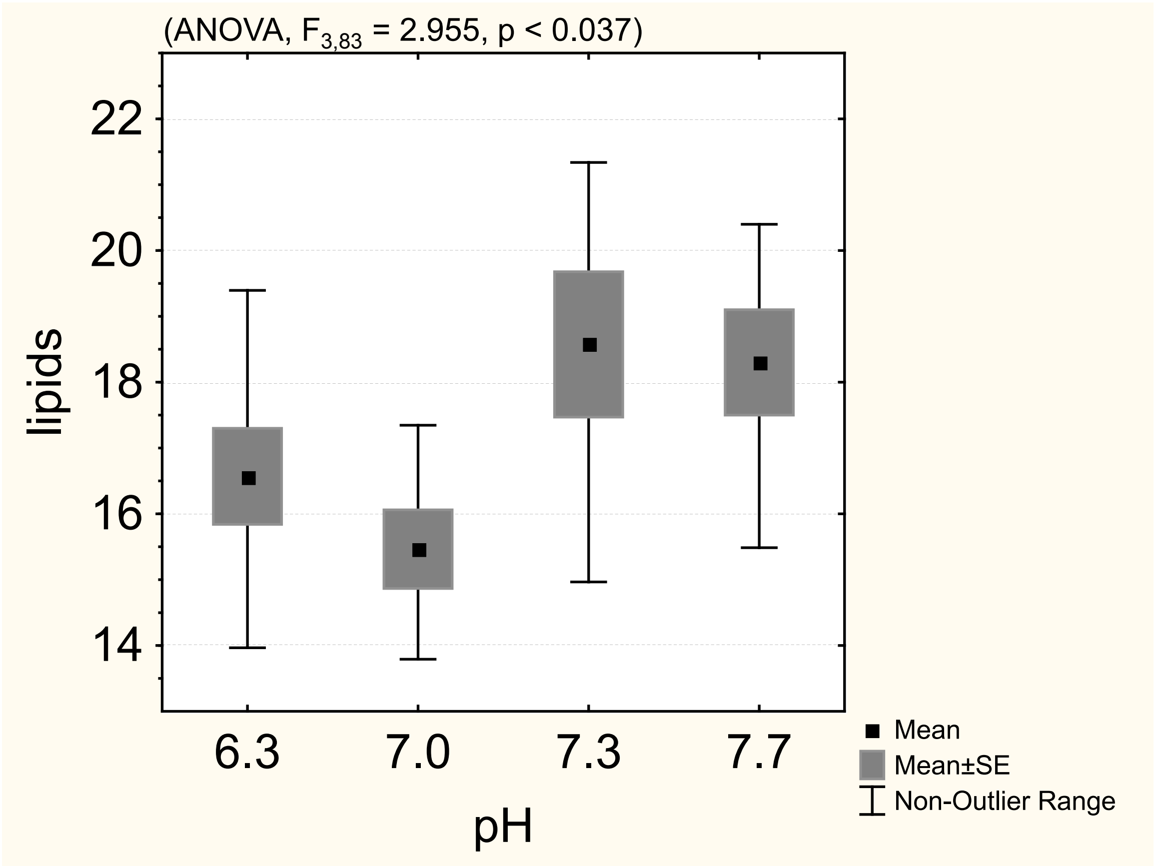
Biological responses

Content of biochemical compounds (lipids, carbohydrates, glycogen, proteins (%)

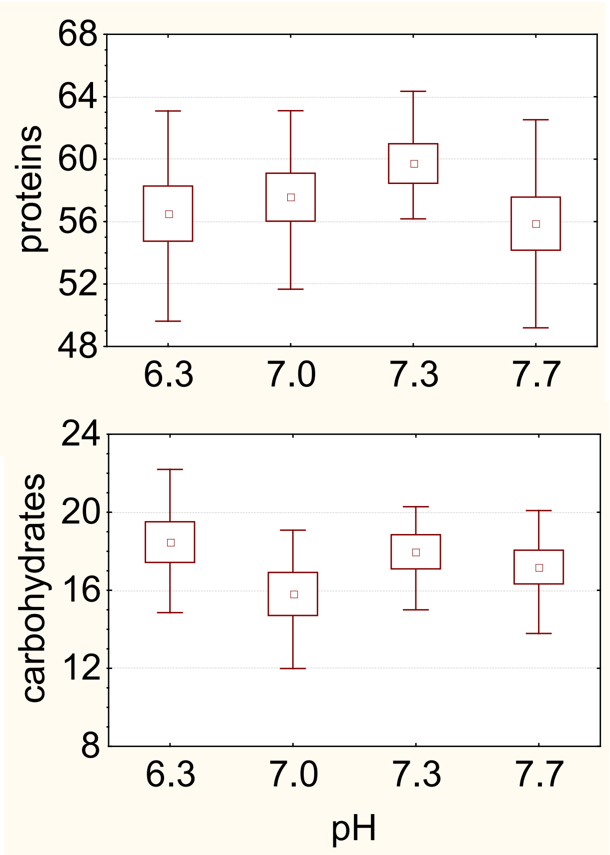


apparent temporal variations with decreased lipids and carbohydrates after 7 days and 6 weeks

Biological responses



(ANOVA, $F_{3,83} = 2.955$, $p < 0.037$)

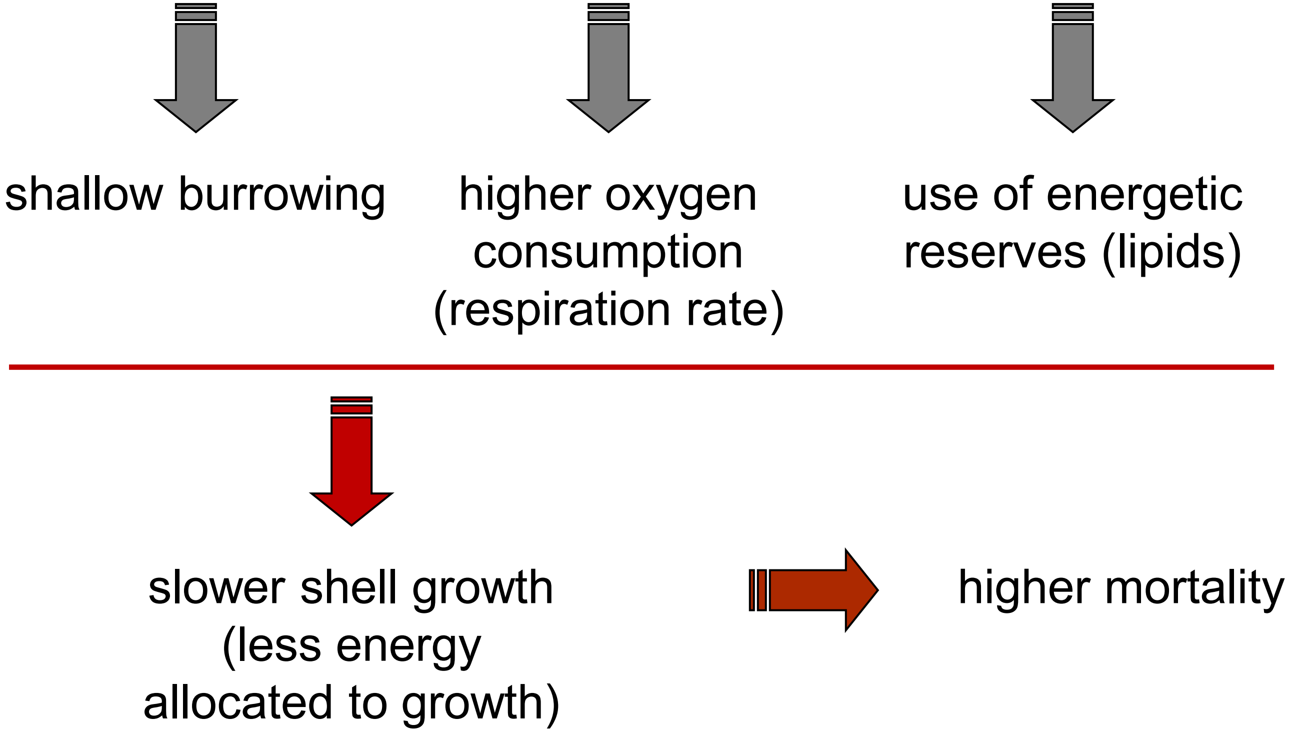


only lipds varied with pH;
under CO_2-stress lipids are used as high energy sourse for compensatory reactions of the bivalves

Conclusions

Increased CO_2 concentration (acidification) affects behavioural and physiological responses of the telinid bivalve *Macoma balthica* from the Baltic Sea.

High CO_2 induces stress → compensatory reactions → higher energy demand



shallow burrowing

higher oxygen consumption (respiration rate)

use of energetic reserves (lipids)

slower shell growth (less energy allocated to growth)

higher mortality

Physiological mechanisms behind bivalve responses at pH 6.3???

Thank you for your attention