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The effect of CO_2-induced seawater acidification on the behaviour and metabolic rate of the Baltic clam *Macoma balthica*

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The aim of this study was to investigate the effect of CO_2-induced seawater acidification (pH 8.1; control pH 7.0 and 6.0) on the survival, behaviour and metabolic rate of the Baltic clam *Macoma balthica* during short- (36 hours) and long-term (14 days) exposures. Total metabolic rate and gaping behaviour (shell opening and closing) were determined based on heat dissipation measurements using an isothermal twin calorimeter of the Calvet type. The survival and burrowing behaviour during long-term exposure were observed every day. Short-term exposure to reduced pH did not significantly affect the resting or active metabolic rate. Neither were there any significant changes in gaping behaviour. However, long-term exposure significantly affected burrowing behaviour. In the lowest-pH treatment, both resting and active metabolic rates were also significantly higher than in the control.

Introduction

Ocean acidification, connected with anthropogenic CO_2 emissions to the atmosphere and its absorption by oceanic waters is a global problem, which may pose a serious threat to marine biodiversity (Doney *et al*. 2009, Hendriks *et al*. 2010). In the Baltic Sea, great seasonal fluctuations of pH and pCO_2 are observed in central parts (Thomas & Schneider 1999, Beldowski *et at*. 2010), but also in highly eutrophicated coastal areas (Thomsen *et al*. 2010, Saderne *et al*. 2013). In the latter, seasonal changes in pH, primarily due to decomposition of excessive amounts of organic matter (almost 1 pH unit; Jakubowska *et al*. 2013, Melzner *et al*. 2013), are considerably greater than the expected annual pH decrease in surface oceanic waters, which is predicted to be 0.002 pH units at the most (Orr 2011). Moreover, relatively low alkalinity of the Baltic Sea results in low buffering capacity against pH changes (Hjalmarsson *et al*. 2008). Consequently, organisms which inhabit coastal areas of the Baltic Sea, especially benthic fauna, are exposed to rapid and severe changes in pH and carbonate chemistry (Omstedt *et al*. 2010). Future changes in eutrophicated coastal areas, which are highly variable systems, are thus hard to predict. It is however known that in the future, the amplitude of the pH seasonal cycle will increase due to intensified biologi-

cal production and mineralization connected with increased nutrient loads (Omstedt *et al*. 2010, 2012). This could lead to much lower pH values than already observed.

In comparison with other invertebrate groups (e.g. crustaceans), bivalves usually have poorly developed ion-exchange mechanisms, thus they are considered to be weak acid–base regulators, which makes them more vulnerable to ocean acidification (Pörtner *et al*. 2004, Thomsen *et al*. 2010, Heinemann *et al*. 2012). Many studies demonstrated that a decrease in seawater pH induced by CO_2 negatively affects different bivalve processes, including hemolymph pH (Lannig *et al*. 2010, Schalkhausser *et al*. 2012), respiration (Michaelidis *et al*. 2005, Cummings *et al*. 2011), clearance (Liu & He 2012, Navarro *et al*. 2013), excretion (Michaelidis *et al*. 2005, Thomsen & Melzner 2010), calcification (Gazeau *et al*. 2007, Ries *et al*. 2009), shell and somatic growth (Beniash *et al*. 2010, Melzner *et al*. 2011), health (Beesley *et al*. 2008), gene expression (Hüning *et al*. 2012), embryonic and larval development (Kurihara *et al*. 2008, Gaylord *et al*. 2011) and survival (Berge *et al*. 2006, Beniash *et al*. 2010). However, responses to acidification are highly species-specific and can significantly vary even between closely-related bivalve taxa (for a review *see* Gazeau *et al*. 2013).

One of the species which is naturally exposed to pH changes is the Baltic clam *Macoma balthica*. This euryhaline tellinid bivalve commonly occurs in marine and estuarine soft-bottom habitats in the northern hemisphere (Bonsdorff & Wenne 1989, Väinölä 2003). In the Baltic Sea, it is considered a key species that dominates the biomass of coastal as well as open sea areas at different depths (Laine 2003, Aarnio *et al*. 2011, Havenhand 2012). *Macoma balthica* being a facultative deposit and suspension feeder (Olafsson 1989, Tallqvist 2001) plays a crucial role in sediment bioturbation and enrichment (Reise 1983, Michaud *et al*. 2006), but it is also able to remove pelagic organic matter when abundant in the water column (Lin & Hines 1994). Moreover, it occupies an important position in the trophic web as a food source for fish (Mattila & Bonsdorff 1998) and invertebrates (Hines *et al*. 1990, Ejdung & Elmgren 2001). Due to its importance, the structure and recruitment success of the Baltic clam population are often used as indicators of environmental conditions (Bonsdorff *et al*. 1995). Furthermore, as evidenced by numerous studies, *M. balthica* has a broad range of tolerance to different unfavourable environmental factors such as oxygen deficiency (e.g. Tallqvist 2001, Long *et al*. 2008) or the presence of hydrogen sulphide (e.g. Janas & Szaniawska 1996, Jahn *et al*. 1997, Janas *et al*. 2007), and is able to inhabit regions rather unattractive to other organisms. Given the above, it is also an interesting model species. Existing data on the effect of seawater acidification on *M. balthica* cover only the early life history and show a significant effect of this factor on fertilization, embryogenesis or larval development, including clams from the Baltic Sea (Jansson *et al*. 2013) as well as from the North Sea (Van Colen *et al*. 2012). Research related to the metabolic rate and behaviour of adult individuals is scarce, also among studies concerning the effect of acidification on other bivalve species. It is noteworthy that both changes in the behaviour (Kramer 1987, Normant & Szaniawska 2000) and metabolic rate or type (Hammerstedt & Loverien 1983, De Zwaan & Putzer 1985) are the first noticeable responses of an organism exposed to environmental stress. Moreover, due to the capability of bivalves to use anaerobic pathways to produce energy under different environmental conditions, it is extremely important to measure their total metabolic rate (Hardewig *et al*. 1991). Direct calorimetry (heat dissipation measurements; Gnaiger 1979, Lamprecht 1998) is the only method that allows to measure both aerobic and anaerobic phases. It is also worth pointing out that direct calorimetry allows not only for measuring the total metabolic rate but also continuous registration of animal behaviour (Lamprecht 1983). However, there are no studies regarding the effect of decreased pH on bivalve metabolism, measured using this method.

Given the above, the objective of our study was to evaluate the effect of CO_2-induced reduced water pH on the gaping behaviour and total metabolic rate of *M. balthica* under short-term exposure (36 hours) and on the survival, burrowing behaviour and total metabolic rate during long-term (14 days) exposure. In the experiments, we used three pH levels: 8.1 for the control, as well as 7.0 and 6.0 to represent reduced pH, the former being close to the

values occurring in many coastal eutrophicated habitats of the Baltic Sea, including the Gulf of Gdańsk, and the latter representing extremely low pH. It is worth pointing out that studies on organisms exposed to pH levels lower than the levels found in their natural environment or projected for near-future climate change scenarios are invaluable. They give a unique opportunity to observe and understand response mechanisms involved in the compensation of pH imbalances, thus shedding light on differences in the sensitivity and tolerance limits of marine invertebrate taxa to ocean acidification (Pörtner 2008, Widdicombe & Spicer 2008, Melzner *et al*. 2009, Whiteley 2011). It may be assumed that due to the poor ability to compensate for acid–base imbalances of body fluids observed in many bivalves, lower pH will affect the metabolic rate of *M. balthica*. Acidification may also reduce activity and affect gaping behaviour of a clam trying to minimise its adverse effects. On the other hand, one can expect that high annual pH amplitudes and values as low as 7.2 (Jakubowska *et al*. 2013) along with resistance to other environmental stressors may induced the development of some adaptations in *M. balthica*.

Material and methods

Collection of animals and their maintenance

Macoma balthica specimens were collected in September 2012 from the Gulf of Gdańsk near the Hel Peninsula (the southern Baltic Sea; 54°35´N, 18°44´E, depth: 51 m). In the laboratory, bivalves were kept for over a week in an aquarium filled with fine-grain sediment and aerated water with parameters close to the *in-situ* conditions (T = 13 °C, S = 7‰, pH ~8.1). We fed the clams with sediment containing 6.47% ± 0.12% dw organic matter (measured by burning three samples at 450 °C for 12 h).

The short-term exposure experiment

Metabolic rate was determined by heat-dissipation measurements conducted in an isothermal twin calorimeter of the Calvet type, with vessels of 24 ml, as described by Normant *et al*. (2007). The calorimeter was equipped with a flow-through system, described by Jakubowska *et al*. (2013), which allows to control and regulate water parameters, including pH. Experimental-water pH was kept at a constant level by a pH controller (ProFlora pH Control, JBL, Germany) attached to a CO_2 cylinder. The experimental water (T = 13 °C, S = 7‰) was pumped through the calorimetric vessels (measuring and the reference) at a rate of 0.2 ml min^{-1}, by a two-channel peristaltic pump (Ecoline VC-MS/CA REGLO Digital, Ismatec, Switzerland). The calorimetric signal was measured at 1-minute intervals by a nanovoltmeter (34420A, Agilent Technologies, USA) with a resolution of 1 nV and registered by a computer in MS Excel. Before the measurements, the calorimeter was calibrated using electrical, 1000 Ω heaters placed inside the vessels. The calibration factor (sensitivity) was 128.80 μV mW^{-1}.

The studies were performed on *M. balthica* (n = 9) with shell length ± SD = 17.29 ± 1.66 mm, tissue dry weight ± SD = 0.04 ± 0.02 g. We placed the bivalves individually in the calorimetric vessel filled with water and 7 g of sterilized (110 °C, 48 h) sediment for 36 hours. During this period, pH was changed every 12 hours, starting from the control (8.1), through 7.0, down to 6.0. The appropriate pH level in the calorimetric vessels was reached after total exchange of water in them which took two hours. Therefore, the first two hours of the measurements were not analysed and not used in calculations for any of the pH treatments.

The specific metabolic rate of a single resting (mean value calculated for the periods between activity peaks) and active (maximum recorded activity) bivalve was calculated and expressed in milliwatts per gram of tissue dry weight (mW g^{-1} dw). The resting rate was the minimal rate, but bivalves were not starved during the measurements (they feed continuously) so we cannot use the term 'standard metabolic rate'. Based on the power–time curves and methods given by Jakubowska and Normant (2015), the mean duration of activity peaks and the ratio between maximal-recorded and resting metabolic rates were calculated for each individual. The

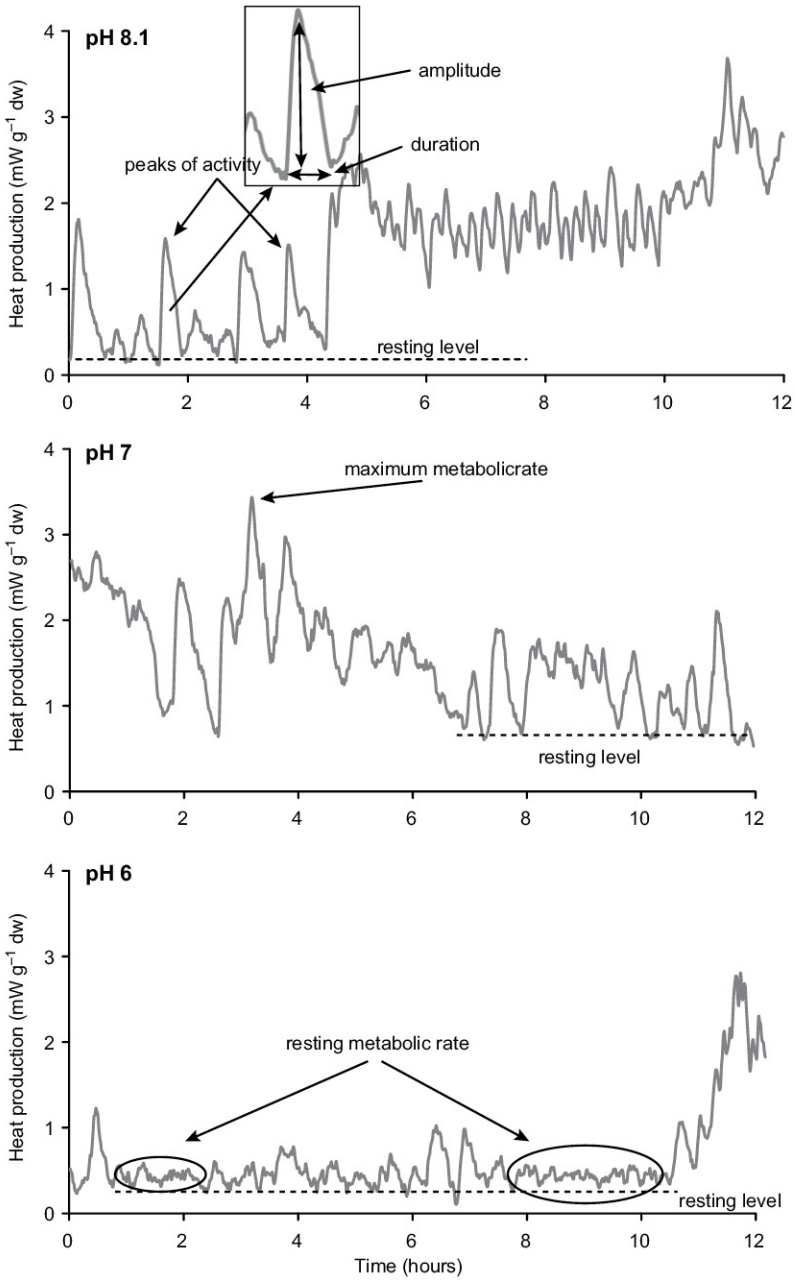

Fig. 1. Sample power-time curves of a single *Macoma balthica* individual (dry weight = 0.053 g) exposed to different pH.

duration of activity peaks was calculated based on the periods between the two low values of the metabolic rate (between which the activity peak occurred; Fig. 1). Only distinct peaks (amplitude greater than 4 μV) were chosen for the calculations. The peaks which began in one pH treatment and ended in another as well as peaks after which metabolism did not return to the resting rate were not used in the calculations. Depending on the activity of the studied bivalves, the number of analysed peaks in one pH treatment varied from 4 to 12 per individual. The durations of all activity peaks calculated for all bivalves in each pH treatment (n = 65 at pH 8.1, n = 57 at pH 7.0, n = 58 at pH 6.0) were averaged and presented as mean ± SD. The mean duration for each bivalve in the studied treatments was also calculated and used for statistical comparisons.

The long-term exposure experiment

We used a flow-through experimental system to study the impact of CO_2-induced water acidification on organisms (described in detail by Jakubowska *et al*. 2013). The setup consisted of a mixing tank (volume 220 l), a tank with non-acidified seawater (volume 220 l) to prevent excessive pH drop, and experimental aquaria (volume 4 l). A peristaltic pump (Ecoline VC-MS/CA 4-12, ISMATEC, Switzerland) was used to maintain the continuous flow of water through the aquaria at a rate of 25 ml per minute. Gaseous CO_2 was introduced into the water in the mixing tank (T = 13 °C, S = 7‰) in the form of small bubbles coming from a perforated tube, until the specified pH level was reached. pH was maintained at a constant level by a pH controller (ProFlora pH Control, JBL, Germany).

We conducted the study on 108 individuals of *M. balthica* (shell length ± SD = 14.61 ± 1.43 mm, tissue dry weight ± SD = 0.02 ± 0.01 g), 36 per pH treatment. There were no significant (Kruskall-Wallis test: $p > 0.05$) differences in shell morphometrics and weights of the studied individuals among the pH treatments. In each experimental pH treatment (8.1 [control], 7.0 and 6.0), three experimental aquaria (12 individuals per aquarium) filled with water and fine-grained sediment were used. Bivalves were gradually acclimated to new pH at a rate of 0.20 pH units per two days, and then exposed to each pH treatment for 14 days. Animals were fed constantly with sediment as during the acclimation period. During this time, the survival rate and burrowing behaviour (the number of individuals on the sediment surface, buried and partially buried in the sediment) were recorded daily. Dead individuals were immediately removed. The water parameters: pH (± 0.01), salinity (± 0.01‰), oxygen concentration (± 0.01 mg l^{-1}) and temperature (± 0.01 °C) were also controlled daily (Multi 340i meter, WTW, Germany) (*see* Table 1). There were no significant differences (Kruskall-Wallis test: $p > 0.05$) in the water parameters (T, S, O_2) among the pH treatment or among the aquaria within each treatment.

After two weeks of exposure, we randomly took ten individuals from each pH treatment for the metabolic rate measurements, which were performed in the calorimeter with the flow-through system as described in the previous section. Bivalves were put individually in a calorimetric vessel and exposed to one experimental pH level (the same as in the two-week exposure) for approximately six hours. The specific metabolic rate of each individual was calculated and expressed in milliwatts per gram of tissue dry weight (mW g^{-1} dw).

Statistical analysis

Friedman's ANOVA was used to test for differences between metabolic rate and the duration of activity peaks at the experimental pH levels during the short-term exposure. The Kruskall-Wallis test was used to test for differences in (1) the morphometric parameters of the studied bivalves, (2) the water parameters during the long-term exposure to different pH treatments, and (3) the survival and metabolic rates at the experimental pH levels during the long-term exposure. After performing the Kruskall-Wallis test and Friedman's ANOVA, *post-hoc* Dunn's test was used to establish which means differ significantly. The burrowing behaviour of *M. balthica* exposed to different pH treatments was compared using a χ^2-test.

Results

The short-term exposure experiment

Bivalves exhibited their natural gaping behaviour which includes periods of rest (closed shell valves and/or hidden siphons) indicated by low values of heat production and periods of increased activity (opened shell valves and/or stretched siphons),

Table 1. Values (mean ± SD) of pH, temperature, salinity and oxygen concentration (14-day averages).

	pH = 8.1	pH = 7.0	pH = 6.0
pH	8.10 ± 0.03	7.01 ± 0.02	6.04 ± 0.03
T (°C)	12.89 ± 0.31	12.89 ± 0.21	12.88 ± 0.19
S (‰)	7.05 ± 0.11	7.05 ± 0.07	7.02 ± 0.06
O_2 (mg O_2 l^{-1})	7.59 ± 0.25	7.65 ± 0.24	7.65 ± 0.34

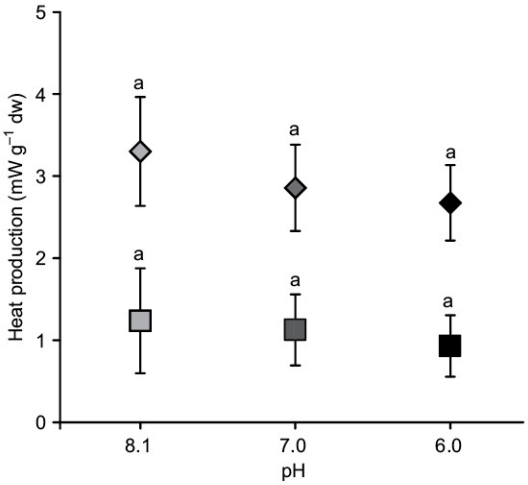

Fig. 2. Resting and active heat production rates (mean ± SD) of *Macoma balthica* during the short-term exposure to three pH levels. The same letters indicate no significant differences at $p < 0.05$; $n = 9$, squares = resting, diamonds = active.

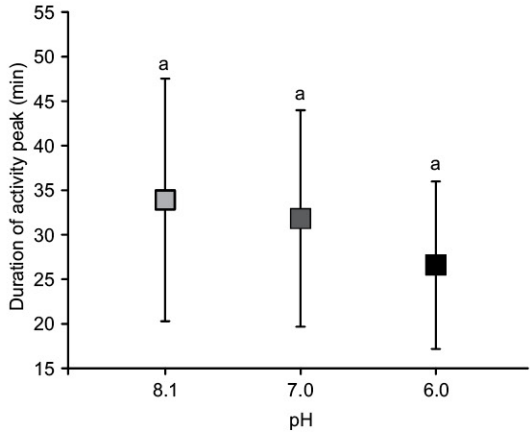

Fig. 3. Duration of enhanced activity peaks (mean ± SD) of *Macoma balthica* during the short-term exposure experiment to different pH. The same letters indicate no significant differences at $p < 0.05$; $n = 9$.

seen as peaks of different heights in the power-time curve (*see* Fig. 1). However, high inter-individual variability in activity, and thus consequently in the metabolic rate was observed. The example shows the most diverse behaviour, thus metabolic rate, which also differs among experimental pH levels. The power–time curves for most of the studied bivalves indicated frequent activity peaks and periods of rest between them, usually at all pH levels.

The short-term exposure to low seawater pH did not cause significant differences (Friedman ANOVA: $\chi^2 = 0.22$, df = 2, $p = 0.895$) in the resting metabolic rate of *M. balthica*, which averaged 1.2 ± 0.6, 1.1 ± 0.4 and 0.9 ± 0.4 mW g^{-1} dw at pH 8.1, 7.0 and 6.0, respectively (Fig. 2). Similarly, there was no significant effect of this factor on the maximum recorded activity (Friedman ANOVA: $\chi^2 = 4.22$, df = 2, $p = 0.121$). Nonetheless, a noticeable reduction of both metabolic levels with decreasing pH was observed. The active (maximum) metabolic rates at pH 8.1, 7 and 6 were on average 3.6 ± 2.8, 2.9 ± 1.2 and 3.2 ± 1.1 times higher than the resting rates at the same pH levels.

The durations of a single activity peak at pH 8.1, 7.0 and 6.0 were 33.9 ± 13.6, 31.8 ± 12.1 and 25.6 ± 9.4 min, respectively (Fig. 3). Despite a noticeable reduction in this parameter with decreasing pH, changes were not significant (Friedman ANOVA: $\chi^2 = 4.22$, df = 2, $p = 0.121$).

The long-term exposure experiment

After two weeks of exposure, the survival of *M. balthica* was significantly lower at pH 6.0 (91.7%) than in other treatments (100% at pH 8.1 and pH 7.0; Kruskal-Wallis test: $H = 39$, $p < 0.001$).

Water pH significantly affected the burrowing behaviour of *M. balthica* (Fig. 4): it was significantly different at pH 7.0 and 6.0 than in the control (χ^2-test: $\chi^2 = 61.29$, $p < 0.001$; and $\chi^2 = 26.29$, $p < 0.001$, respectively).

The long-term exposure to reduced pH showed that the metabolic rate of *M. balthica* increases with decreasing pH (Fig. 5). The averaged resting rate of this process was 1.5 times higher at pH 7.0 and 2.5 times higher at pH 6.0 than in the control treatment (Kruskal-Wallis test: $H = 8.75$, $p = 0.013$). However, a significant difference was found only between the 8.1 and 6.0 pH treatments (Dunn's test: $p = 0.011$). Similarly, the maximum recorded activity was significantly higher than in the control only at pH 6.0 (Kruskal-Wallis test: $H = 7.23$, $p = 0.027$; Dunn's test, $p = 0.022$). The averaged active rate was 1.3 times higher at pH 7.0 and 1.7 times higher at pH 6.0 than at pH 8.1. The

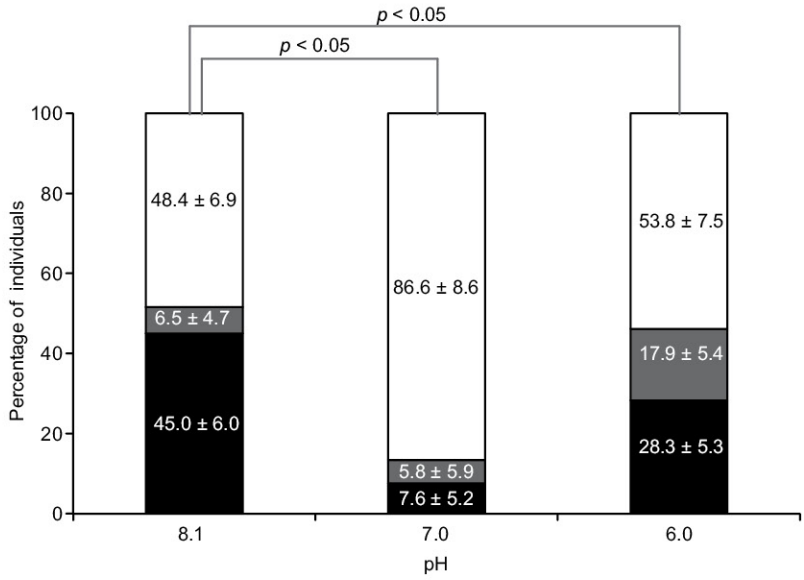

Fig. 4. Mean percentages of individuals of *M. balthica*: buried (white), partially buried (grey) and on the sediment (black) surface during 14-day exposure to three pH levels; $n = 36$.

active-to-resting-rate ratios were 3.5 ± 1.9, 3.4 ± 2.7, 7.0 and 2.0 ± 0.5, at pH 8.1, 7.0 and 6.0, respectively.

Discussion

Our study provides the first information on the responses of adult individuals of *M. balthica* to CO_2-induced reduction in water pH. It revealed that the Baltic clam can adapt to extremely low pH, lower than the values that may be expected by the end of this century due to ongoing ocean acidification, but it is energetically more costly.

Although the values of resting metabolic rate of *M. balthica* decreased with decreasing pH during the short-term exposure to acidification, the differences were not significant probably due to considerable inter-individual variation (cf. SD values). This is not surprising as high intraspecific variation in activity and consequently in the total metabolic rate was also found in other, especially infaunal, bivalve species during calorimetric studies (Pedersen 1992, Holopainen & Penttinen 1993). Infaunal bivalves are considered to exhibit a wider range of inter-individual variability in heat dissipation in both favourable and adverse environmental conditions than epifaunal species usually showing periodic fluctuations in their metabolic rate as a result of continuous water pumping (Taylor & Brand 1975, Wang & Widdows 1993). Unchanged standard or resting metabolic rates were observed in dif-

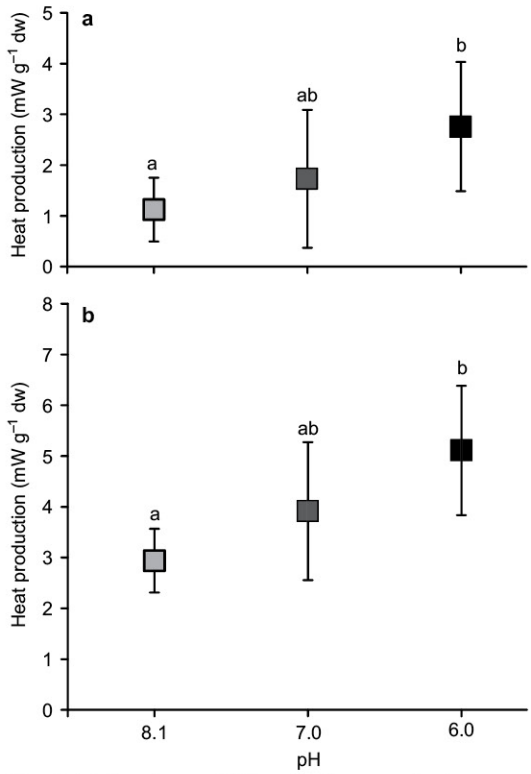

Fig. 5. (**a**) Resting and (**b**) active heat production rates (mean ± SD) of *M. balthica* after the long-term exposure to different pH. Different letters indicate significant differences at $p < 0.05$; $n = 10$.

ferent bivalve species, e.g. *Pinctada fucata* (Liu & He 2012), *Crassostrea gigas* (Lanning *et al*. 2010) and *Pecten maximus* (Schalkhausser *et al*. 2012), although during longer (several days) exposures to acidification, but also in *Mytilus edulis trossulus* after 24 hours (Jakubowska & Normant 2015). However in our study, resting metabolism decreased (insignificantly) by 25% at the lowest pH level already after 24 h, which should be considered an initial response to the studied factor. Despite the fact that most of the regulatory mechanisms (e.g. bicarbonate uptake to maintain high haemolymph pH) are activated almost immediately after exposure to low pH, complete pH restoration is usually achieved after a longer period (few hours to few days), depending on the regulatory abilities of each taxa and the pH value (Cameron 1978, Hayashi *et al*. 2004, Dupont & Thorndyke 2012). Thus, the noticeable metabolic response (fully compensated depression) is an even more delayed process. An interesting situation was observed by Hammer *et al*. (2011) in the deep-sea bivalve *Acestea excavate*, in which oxygen consumption decreased during the initial phase of hypercapnia, but approached the control values after 92 hours. *Macoma balthica* probably also requires a longer period at pH as low as 6.0 for the metabolic rate to return to the normal level.

The opposite situation was observed by us in *M. balthica* after two weeks of exposure when the resting metabolic rate increased with decreasing pH. This increase most probably resulted from the additional cost of acid–base regulation and compensatory increases in the expression of biomineralization-related enzymes (Beniash *et al*. 2010, Comeau *et al*. 2010, Thomsen & Melzner 2010). As found and concluded by Stumpp *et al*. (2011a, 2011b), the gene expression of Na^+/K^+-ATPase could be upregulated under hypercapnia, because its activity increases and it can account for even more than 70% of the metabolic rate. Therefore, the increased metabolic rate can be responsible for high resilience to elevated pCO_2 and should be considered a key process involved in the acclimation or adaptation to this factor (Parker *et al*. 2012). However, the higher cost of maintenance results in less energy available for locomotion, growth and reproduction. Moreover, if an organism needs to increase energy expenditure on maintenance, it apparently functions in stressful conditions. Our results correspond with those reported for several other bivalve species. Elevated respiration rates at reduced pH were observed in *Laternula elliptica* (Cummings *et al*. 2011), *Mytilus edulis* (Thomsen & Melzner 2010), *Crassostrea gigas* (only at elevated temperatures; Lannig *et al*. 2010), *Crassostrea virginica* (also connected with higher ADP levels; Beniash *et al*. 2010) and *Saccostrea glomerata* (Parker *et al*. 2012). However, in a few bivalve species the reverse reaction, i.e. a significant decrease in the metabolic rate with decreasing seawater pH was observed (e.g. Michalidis *et al*. 2005, Liu & He 2012, Navarro *et al*. 2013). This might be primarily connected with their poor ability to compensate for acid–base changes during hypercapnia since the decrease in extracellular pH is the main cause of metabolic depression (Reipschläger & Pörtner 1996). Metabolic suppression is also regarded as a time-limited adaptive strategy to minimize energy expenditure and to extend the period during which an organism can withstand adverse environmental conditions (Guppy & Withers 1999). It should be emphasized, however, that both metabolic depressions and increments result in trade-offs in energy allocation between different processes.

The lack of metabolic depression and generally high tolerance of *M. balthica* to acidification could be due to some effective acid–base compensation abilities. The increase in HCO_3^- concentration in the hemolymph is a primary mechanism of compensation for extracellular pH disturbances in different marine animals (Pörtner *et al*. 2004). As demonstrated by several studies, shelled molluscs are able to obtain bicarbonates from passive shell dissolution, which enables them to compensate at least partially a hemolymph pH decrease during water acidification (e.g. Michaelidis *et al*. 2005, Marchant *et al*. 2010). This mechanism is also observed during shell closure and body acidosis related to anaerobic metabolism and its products during exposure to other unfavourable conditions (Burnett 1988). In *M. balthica*, this process and at least partial compensation of extracellular pH changes might occur. In our study, however, hemolymph pH and ionic composition were not measured due

to a relatively small tissue size, thus we were unable to properly collect hemolymph. Also during shell closure, bivalves use the oxygen remaining in the cells and hemolymph, which results in an increase in pCO_2 due to limited gas exchange with the surroundings (Booth *et al.* 1984). Moreover, metabolic acids are produced during anaerobic metabolism, which also causes respiratory acidosis (Burnett 1988). However, during long-term adaptations to hypoxia, the amount of protons and the rate of their accumulation are decreased, which — supported by the reduced anaerobic metabolism — allows for long-term survival (Pörtner 1987). This kind of adaptation may also have contributed to elevated tolerance of *M. balthica* to low pH of body fluids. In the natural environment, this species often encounters oxygen deficiency and other unfavourable conditions, which may result in shell closure and the necessity to use anaerobic pathways. Moreover, the increased tolerance to acidification could also result from food abundance in the environment. Melzner *et al.* (2011) and Thomsen *et al.* (2013) reported that elevated nutrient concentrations in the Baltic Sea enable *M. edulis* to obtain sufficient energy for somatic and shell growth, and to sustain high metabolic rates and consequently dominate in the highly-acidified environment. Also *Macoma balthica* can benefit from eutrophication in a similar way (Beukema *et al.* 1996).

On the other hand, the increased metabolic rates could also be a result of reduced tolerance to higher temperatures. A negative synergistic effect is expected if both factors increase metabolic rate. A few studies have already demonstrated that sensitivity to acidification is temperature-dependent — the highest metabolic rates were found when molluscs were exposed to elevated pCO_2 and temperature (Comeau *et al.* 2010, Lannig *et al.* 2010). Ocean acidification also narrows the thermal tolerance in bivalves (Schalkhausser *et al.* 2012) and crabs (Metzger *et al.* 2007, Walther *et al.* 2009). It seems to be a serious threat in relation to global warming — another problem which co-occurs and affects the physiology of marine organisms simultaneously with ocean acidification (Pörtner 2008). This should be taken into consideration in further studies on *M. balthica*, because despite its resistance to many stressors, it has a relatively low tolerance to elevated temperatures (Beukema *et al.* 2009). However, the negative effect of climate-change-related temperature rise will be rather restricted to the species' southern limit (vicinity of Gironde estuary) where Baltic clams have higher metabolic rates (Jansen *et al.* 2007) and are generally regarded most sensitive to stress (Hummel *et al.* 2000).

The active (maximum) metabolic rate changed in the same manner as the resting rate; a slight insignificant decrease was observed during the short-term exposure, but after two weeks at pH 6.0, it significantly increased as compared with that in the control. During the short-term exposure to reduced pH, both metabolic rates remained almost unchanged, thus the ratios between those two levels were similar. However, after longer exposure, the resting metabolism at pH 6.0 increased more than the active one, which resulted in their a lower ratio. This situation might be associated with some relocation of energy from activity to, for example, acid–base regulation. A similar situation was observed by Schalkhausser *et al.* (2012) in *Pecten maximus* in which the difference between the maximum and resting aerobic metabolic rates was reduced during acidification which was connected with a reduced clapping force. Nonetheless, even at the lowest pH during short- and long-term exposures, *M. balthica* remained active. Moreover, the duration of activity peaks during the short-time exposure was not affected by lower pH. The peaks lasted for ~30 minutes, were frequent and recurrent, which means that *M. balthica* relied on aerobic metabolism and behaved naturally. Valve movement responses are fast behavioural reactions of bivalves, thus are considered a useful tool in biological monitoring of natural waters (Kramer *et al.* 1989). When bivalves close their shells for a longer period and reduce their activity, it is equivalent to reduced standard metabolism, feeding and respiration, and is considered an indicator of stress conditions (Ortmann & Greishaber 2003, García-March *et al.* 2008). The ability to maintain aerobic metabolism during acidification should be considered an adaptation that enables them to sustain basic physiological functions.

Yet another behavioural parameter used in bivalves (infaunal species) as an indicator of

stress conditions is burrowing activity. However, a surprising situation was observed by us: namely, burrowing activity at pH 6.0 was more similar to the control than that at pH 7.0. In all the treatments, feeding traces were observed on the sand, indicating so-called crawling behaviour of emerged individuals, whereas the burrowed ones often extended their siphons above the sediment surface. As compared with suspension-feeding Baltic clams, deposit feeders usually decrease their burying depths to increase the area that can be reached by their inhalant siphons (Olaffson 1986, Zwarts & Wanink 1989, Lin & Hines 1994). They also exhibit a crawling behaviour which facilitates food intake at the expense of greater energy expenditure (Mouritsen 1997). It should be kept in mind that feeding mode, thus burrowing or crawling behaviour, is dependent not only on the suspended food availability but also on other factors like predator pressure (Piersma *et al*. 1995), sex and growth rates (Mouritsen 1997). Thus, according to available data and as shown in the control treatment, both burying and crawling behaviours should be considered natural and typical for Baltic clams. When analysing burrowing behaviour it should be kept in mind that *M. balthica* can burry themselves in the sediment to the depths exceeding 35 cm (Hines & Comtois 1985). As a result, this species may periodically experience conditions (e.g. pH) much different than in the water column or near the bottom. It is generally known that fluctuations in CO_2 and pH within marine sediments, even in their surface layers, are considerably higher than in the overlaying water. Therefore, it seems reasonable to assume that infaunal species would have an enhanced tolerance to hypercapnia (Pörtner *et al*. 2004, Widdicombe & Spicer 2008). pH as low as 6.5–7.0 were also found in interstitial waters in the Gulf of Gdańsk (Białkowska & Bolałek 2000, Bolałek & Frankowski 2003, Łukawska-Matuszewska *et al*. 2009). During exposure to pH 7.0 the percentage of buried individuals rose to 86%, the value for the control being 48%. The reduced burying depth in *M. balthica* and prolonged residence on the sediment surface were usually observed as a response to unfavourable conditions such as hypoxia in the water and sediment (Tallqvist 2001, Long *et al*. 2008) and high concentrations of heavy metals (Eldon & Kristoffersson 1978, Sokolowski *et al*. 1999). Emerging from the sediment increases predation risk (Lin and Hines 1994, Long *et al*. 2008), therefore, the increased burrowing of *M. balthica* observed at 7.0 pH might be considered positive. However, it is not entirely clear why more calms were buried at pH 7.0 than at pH 6.0. A relatively high percentage of crawling individuals at the lowest pH might mean that *M. balthica* does not need to significantly decrease its activity and conserve energy during long-term and severe water acidification as foraging by crawling demands more energy than extending the siphon during burial. Crawling might rather reflect increased energy demands due to higher costs of maintenance at pH 6.0 as indicated by increased metabolic rates. On the one hand, similarly to that at pH 7.0, the percentage of emerged bivalves at pH 6.0 was lower than in the control, but on the other hand additional metabolic costs resulted in attempts to emerge in order to increase food intake. Unfortunately, there are no studies on the behavioural responses of bivalves to ocean acidification, which makes comparisons of the obtained results difficult.

The fact that *M. balthica* was able to remain active — as evidenced by the burrowing and crawling behaviours, but also by the unaffected gaping activity and the ability to maintain a high metabolic rate — was probably the reason for high survival during 14-day exposure to lowered pH. However, its survival was significantly lower at pH 6 as compared with that in the control treatment, but still was more than 90%, which means that only 3 out of 36 bivalves died (one of them died at the beginning of the experiment most probably due to reasons other than low pH). A similar situation was observed in other bivalves: e.g. survival of *Acesta excavate* was unaffected after 96 hours at pH 6.35 (Hammer *et al*. 2011), or that of *Pecten maximus* after 60 days at pH 7.65 (Schalkhausser *et al*. 2012), even though changes in some physiological processes were evident. It should be kept in mind, however, that survival rates are highly dependent on many factors, mainly duration of exposure, pH (or pCO_2), and are strongly species-specific.

In a broader context, *M. balthica* populations might be severely affected by acidification. Few

investigations showed that even a slight drop in water pH reduces the survival of larval stages and their growth (Van Colen *et al*. 2012, Jansson *et al*. 2013). It is generally known that as compared with adults, larvae and juveniles are usually more sensitive to unfavourable environmental conditions such as acidification. This also applies to *M. balthica* whose adults are considered quite tolerant to different stressors for some time, whereas juveniles are sensitive to even small disturbances in the environment (Bonsdorff *et al*. 1995). However, as demonstrated by Parker *et al*. (2012), acclimation of adult bivalves (*Saccostrea glomerata*) to high pCO_2 during reproduction has positive effects on the resilience of their larvae. Therefore, there is a chance that a similar adaptation might be present or might develop over time in *M. balthica* populations, especially at sites characterized by eutrophication-induced acidification.

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References

Aarnio, K., Mattila, J., Törnroos, A. & Bonsdorff, E. 2011: Zoobenthos as an environmental quality element: the ecological significance of sampling design and functional traits. — *Marine Ecology* 32: 58–71.

Beesley, A., Lowe, D. M., Pascoe, C. A. & Widdicombe, S. 2008: Effects of CO_2-induced seawater acidification on the health of *Mytilus edulis*. — *Climate Research* 37: 215–225.

Beldowski, J., Löffler, A., Schneider, B. & Joensuu L. 2010: Distribution and biogeochemical control of total CO_2 and total alkalinity in the Baltic Sea. — *Journal of Marine Systems* 81: 252–259.

Beniash, E., Ivanina, A., Lieb, N. S., Kurochkin, I. & Sokolova, I. M. 2010: Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. — *Marine Ecology Progress Series* 419: 95–108.

Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T. & Øxnevad S. 2006: Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. — *Chemosphere* 62: 681–687.

Beukema, J. J., Dekker, R. & Jansen, J. M. 2009: Some like it cold: populations of the tellinid bivalve *Macoma balthica* (L.) suffer in various ways from a warming climate. — *Marine Ecology Progress Series* 384: 135–145.

Beukema, J. J., Essink, K. & Michaelis, H. 1996: The geographic scale of synchronized fluctuation patterns in zoobenthos populations as a key to underlying factors: climatic or man-induced. — *ICES Journal of Marine Science* 53: 964–971.

Białkowska, I. & Bolałek, J. 2000: Distribution of the Fe (II) and Fe (III) dissolved in the interstitial water of the Gulf of Gdańsk. — *Oceanological Studies* 29: 43–54.

Bolałek, J. & Frankowski, L. 2003: Selected nutrients and iron in interstitial waters of the Estuary of Southern Baltic (Gulf of Gdańsk and the Pomeranian Bay) in relation to redox potential. — *Water, Air, and Soil Pollution* 147: 39–50.

Bonsdorff, E., Norkko, A. & Boström, C. 1995: Recruitment and population maintenance of the bivalve *Macoma balth*ica (L.) — factors affecting settling success and early survival on shallow sandy bottoms. — In: Eleftheriou, A., Ansell, A. D. & Smith, C. J. (eds.), *Biology and Ecology of Shallow Coastal Waters. Proceedings of the 28th European Marine Biology Symposium*: 253–260. Olsen & Olsen, Fredensborg.

Bonsdorff, E. & Wenne, R. 1989: A comparison of condition indices of *Macoma balthica* (L.) from the northern and southern Baltic Sea. — *Netherlands Journal of Sea Research* 23: 45–55.

Booth, C. E., McDonald, D. G. & Walsh, P. J. 1984: Acid-base balance in the sea mussel, *Mytilus edulis*. I: Effects of hypoxia and air-exposure on hemolymph acid-base status. — *Marine Biology Letters* 5: 347–358.

Burnett, L. E. 1988. Physiological responses to air exposure: acid-base balance and the role of branchial water stores. — *American Zoologist* 28: 125–135.

Cameron, J. N. 1978: Effects of hypercapnia on blood acid-base status, NaCl fluxes, and trans-gill potential in freshwater blue crabs, *Callinectes sapidus*. — *Journal of Comparative Physiology* 123: 137–141.

Comeau, S., Jeffree, R., Teyssié, J. L. & Gattuso, J. P. 2010: Response of the Arctic pteropod *Limacina helicina* to projected future environmental conditions. — *PLoS ONE* 5: e11362, doi:10.1371/journal.pone.0011362.

Cummings, V., Hewitt, J., Van Rooyen, A., Currie, K., Beard, S., Thrush, S. Norkko, J., Barr, N., Heath, P., Halliday, N. J., Sedcole, R., Gomez, A., McGraw, C. & Metcalf, V. 2011: Ocean acidification at high latitudes: potential effects on functioning of the Antarctic bivalve *Laternula elliptica*. — *PLoS ONE* 6: e16069, doi:10.1371/journal.pone.0016069.

De Zwaan, A. & Putzer, V. 1985: Metabolic adaptations of intertidal invertebrates to environmental hypoxia (a comparison of environmental anoxia to exercise anoxia). — *Symposia of the Society for Experimental Biology* 39: 33–62.

Doney, S. C., Fabry, V. J., Feely, R. A. & Kleypas, J. A. 2009:

Ocean acidification: the other CO_2 problem. — *Marine Science* 1: 169–192.

Dupont, S. & Thorndyke, M. 2012: Relationship between CO_2-driven changes in extracellular acid-base balance and cellular immune response in two polar echinoderm species. — *Journal of Experimental Marine Biology and Ecology* 424–425: 32–37.

Ejdung, G. & Elmgren, R. 2001: Predation by the benthic isopod *Saduria entomon* on two Baltic Sea deposit-feeders, the amphipod *Monoporeia affinis* and the bivalve *Macoma balthica*. — *Journal of Experimental Marine Biology and Ecology* 266: 165–179.

Eldon, J. & Kristoffersson, R. 1978: Factors affecting the burrowing activity of *Macoma balthica* (L.). — *Annales Zoologici Fennici* 15: 127–131.

García-March, J. R., Sanchís Solsona, M. Á. & García-Carrascosa, A. M. 2008: Shell gaping behaviour of *Pinna nobilis* L., 1758: circadian and circalunar rhythms revealed by in situ monitoring. — *Marine Biology* 153: 689–698.

Gaylord, B., Hill, T. M., Sanford, E., Lenz, E. A., Jacobs, L. A., Sato, K. N., Russell, A. D. & Hettinger, A. 2011: Functional impacts of ocean acidification in an ecologically critical foundation species. — *Journal of Experimenal Biology* 214: 2586–2594.

Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J. P., O'Connor, W. A., Martin, S., Pörtner, H. O. & Ross, P. M. 2013: Impacts of ocean acidification on marine shelled molluscs. — *Marine Biology* 160: 2207–2245.

Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J. P., Middelburg, J. J. & Heip, C. H. R. 2007: Impact of elevated CO_2 on shellfish calcification. — *Geophysysical Research Letter* 34: L07603, doi:10.1029/2006GL028554.

Gnaiger, E. 1979: Direct calorimetry in ecological energetics. Long term monitoring of aquatic animals. — *Experientia Supplementum* 37: 155–165.

Guppy, M. & Withers, P. 1999: Metabolic depression in animals: physiological perspectives and biochemical generalizations. — *Biological Reviews of the Cambridge Philosophical Society* 74: 1–40.

Hammer, K. M., Kristiansen, E. & Zachariassen, K. E. 2011: Physiological effects of hypercapnia in the deep-sea bivalve *Acesta excavata* (Fabricius, 1779) (Bivalvia; Limidae). — *Marine Environmental Research* 72: 135–142.

Hammerstedt, R. H. & Loverien, R. E. 1983: Calorimetric techniques for metabolic studies of cells and organisms under normal conditions and stress. — *Journal of Experimental Zoology* 228: 459–469.

Hardewig, I., Addink, A. D. F., Grieshaber, M. K., Pörtner, H. O. & van den Thillart, G. 1991: Metabolic rates at different oxygen levels determined by direct and indirect calorimetry in the oxyconformer *Sipunculus nudus*. — *Journal of Experimental Biology* 157: 143–160.

Havenhand, J. N. 2012: How will ocean acidification affect Baltic Sea ecosystems? An assessment of plausible impacts on key functional groups. — *Ambio* 41: 637–644.

Hayashi, M., Kita, J. & Ishimatsu, A. 2004: Acid-base responses to lethal aquatic hypercapnia in three marine fishes. — *Marine Biology* 144: 153–160.

Heinemann, A., Fietzke, J., Melzner, F., Böhm, F., Thomsen, J., Garbe-Schönberg, D. & Eisenhauer, A. 2012: Conditions of *Mytilus edulis* extracellular body fluids and shell composition in a pH-treatment experiment: acid-base status, trace elements and $\delta^{11}B$. — *Geochemistry, Geophysics, Geosystems* 13, Q01005, doi:10.1029/2011GC003790.

Hendriks, I. E., Duarte, C. M. & Álvarez, M. 2010. Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. — *Estuarine, Coastal and Shelf Science* 86: 157–164.

Hines, A. H. & Comtois, K. L. 1985: Vertical distribution of infauna in sediments of a subestuary of central Chesapeake Bay. — *Estuaries* 8: 296–304.

Hines, A. H., Haddon, A. M. & Wiechert, L. A. 1990: Guild structure and foraging impact of blue crabs and epibenthic fish in a subestuary of Chesapeake Bay. — *Marine Ecology Progress Series* 67: 105–126.

Hjalmarsson, S., Wesslander, K., Anderson, L. G., Omstedt, A., Perttilä, M. & Mintrop, L. 2008: Distribution, long-term development and mass balance calculation of total alkalinity in the Baltic Sea. — *Continental Shelf Research* 28: 593–601.

Holopainen, I. J. & Penttinen, O. P. 1993: Normoxic and anoxic heat output of the freshwater bivalves *Pisidium* and *Sphaerium*. — *Oecologia* 93: 215–223.

Hummel, H., Bogaards, R. H., Bachelet, G., Caron, F., Sola, J. C. & Amiard-Triquet, C. 2000: The respiratory performance and survival of the bivalve *Macoma balthica* (L.) at the southern limit of its distribution area: a translocation experiment. — *Journal of Experimental Marine Biology and Ecology* 251: 85–102.

Hüning, A. K., Melzner, F., Thomsen, J., Gutowska, M. A., Krämer, L., Frickenhaus, S., Rosenstiel, P., Pörtner, H. O., Philipp, E. E. R. & Lucassen, M. 2012: Impacts of seawater acidification on mantle gene expression patterns of the Baltic Sea blue mussel: implications for shell formation and energy metabolism. — *Marine Biology* 160: 1845–1861.

Jahn, A., Janas, U., Theede, H. & Szaniawska, A. 1997: Significance of body size in sulphide detoxification in the Baltic clam *Macoma balthica* (Bivalvia, Tellinidae) in the Gulf of Gdansk. — *Marine Ecology Progress Series* 154: 175–183.

Jakubowska, M., Jerzak, M., Normant, M., Burska, D. & Drzazgowski, J. 2013: Effect of the carbon dioxide-induced water acidification on physiological processes of Baltic isopod *Saduria entomon*. — *Journal of Shellfish Research* 32: 825–834.

Jakubowska, M. & Normant, M. 2015: Metabolic rate and activity of blue mussel *Mytilus edulis trossulus* under short-term exposure to carbon dioxide-induced water acidification and oxygen deficiency. — *Marine and Freshwater Behaviour and Physiology* 48: 25–39.

Janas, U., Nowodworska, E. & Bezdzietny, I. 2007: Fitness and chemical composition of the Baltic clam *Macoma balthica* (Linnaeus, 1758) from sulphidic habitats in the Gulf of Gdańsk (Southern Baltic). — *Thermochimica acta* 458: 112–117.

Janas, U. & Szaniawska, A. 1996: The influence of hydrogen

sulphide on macrofaunal biodiversity in the Gulf of Gdansk. — *Oceanologia* 38: 127–142.

Jansen, J. M., Pronker, A. E., Kube, S., Sokolowski, A., Sola, J. C., Marquiegui, M. A., Schiedek, D., Wendelaar Bonga S., Wolowicz, M. & Hummel, H. 2007: Geographic and seasonal patterns and limits on the adaptive response to temperature of European *Mytilus* spp. and *Macoma balthica* populations. — *Oecologia* 154: 23–34.

Jansson, A., Norkko, J. & Norkko, A. 2013: Effects of reduced pH on *Macoma balthica* larvae from a system with naturally fluctuating pH-dynamics. — *PLoS ONE* 8: e68198, doi:10.1371/journal.pone.0068198.

Kramer, D. L. 1987: Dissolved oxygen and fish behaviour. — *Environmental Biology of Fishes* 18: 81–92.

Kramer, K. J., Jenner, H. A. & de Zwart, D. 1989: The valve movement response of mussels: a tool in biological monitoring. — *Hydrobiologia* 188–189: 433–443.

Kurihara, H., Asai, T., Kato, S. & Ishimatsu, A. 2008: Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. — *Aquatic Biology* 4: 225–233.

Laine, A. O. 2003: Distribution of soft-bottom macrofauna in the deep open Baltic Sea in relation to environmental variability. — *Estuarine, Coastal and Shelf Science* 57: 87–97.

Lamprecht, I. 1983: Application of calorimetry to different biological fields and comparison with other methods. — *Bollettino della Società dei Naturalisti in Napoli* 92: 515–542.

Lamprecht, I. 1998: Monitoring metabolic activities of small animals by means of microcalorimetry. — *Pure and Applied Chemistry* 70: 695–700.

Lannig, G., Eilers, S., Pörtner, H. O., Sokolova, I. M. & Bock, C. 2010: Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*-changes in metabolic pathways and thermal response. — *Marine Drugs* 8: 2318–2339.

Lin, J. & Hines A. H. 1994: Effects of suspended food availability on the feeding mode and burial depth of the Baltic clam, *Macoma balthica*. — *Oikos* 69: 28–36.

Liu, W. & He, M. 2012: Effects of ocean acidification on the metabolic rates of three species of bivalve from southern coast of China. — *Chinese Journal of Oceanology and Limnology* 30: 206–211.

Long, W. C., Brylawski, B. J. & Seitz, R. D. 2008: Behavioural effects of low dissolved oxygen on the bivalve *Macoma balthica*. — *Journal of Experimental Marine Biology and Ecology* 359: 34–39.

Łukawska-Matuszewska, K., Burska, D. & Niemirycz, E. 2009: Toxicity assessment by Microtox® in sediments, pore waters and sediment saline elutriates in the Gulf of Gdansk (Baltic Sea). — *CLEAN — Soil, Air, Water* 37: 592–598.

Marchant, H. K., Calosi, P. & Spicer, J. I. 2010: Short-term exposure to hypercapnia does not compromise feeding, acid-base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage. — *Journal of the Marine Biological Association of the United Kingdom* 90: 1379–1384.

Mattila, J. & Bonsdorff, E. 1998: Predation by juvenile flounder (*Platichthys flesus* L.): a test of prey vulnerability, predator preference, switching behaviour and functional response. — *Journal of Experimental Marine Biology and Ecology* 227: 221–236.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M. & Pörtner, H. O. 2009: Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? — *Biogeosciences* 6: 2313–2331.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N. & Gutowska, M. A. 2011: Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. — *PLoS ONE* 8: e24223, doi:10.1371/journal.pone.0024223.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A. Gutowska, M. A., Bange, H. W., Hansen, H. P. & Körtzinger, A. 2013: Future ocean acidification will be amplified by hypoxia in coastal habitats. — *Marine Biology* 160: 1875–1888.

Metzger, R., Sartoris, F. J., Langenbuch, M. & Pörtner, H. O. 2007: Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. — *Journal of Thermal Biology* 32: 144–151.

Michaelidis, B., Ouzounis, C., Paleras, A. & Pörtner, H. O. 2005: Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. — *Marine Ecology Progress Series* 293: 109–118.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B. & Stora, G. 2006: The functional group approach to bioturbation: II. The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment–water interface. — *Journal of Experimental Marine Biology and Ecology* 337: 178–189.

Mouritsen, K. N. 1997: Crawling behaviour in the bivalve *Macoma balthica*: the parasite-manipulation hypothesis revisited. — *Oikos* 79: 513–520.

Navarro, J. M., Torres, R., Acuña, K., Duarte, C., Manriquez, P. H., Lardies, M., Lagos, N. A., Vargas C. & Aguilera, V. 2013: Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. — *Chemosphere* 90: 1242–1248.

Normant, M., Dziekoński, M., Drzazgowski, J. & Lamprecht, I. 2007: Metabolic investigations of aquatic organisms with a new twin heat conduction calorimeter. — *Thermochimica Acta* 458: 101–106.

Normant, M. & Szaniawska, A. 2000: Behaviour, survival and glycogen utilisation in the Baltic isopod *Saduria entomon* exposed to long-term oxygen depletion. — *Marine and Freshwater Behaviour and Physiology* 33: 100–111.

Olafsson, E. B. 1986: Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. — *Journal of Animal Ecology* 55: 517–526.

Olafsson, E. B. 1989: Contrasting influences of suspension-

feeding and deposit-feeding populations of *Macoma balthica* on infaunal recruitment. — *Marine Ecology Progress Series* 55: 171–179.

Omstedt, A. M., Edman, M., Anderson, L. G. & Laudon, H. 2010: Factors influencing the acid-base (pH) balance in the Baltic Sea: a sensitivity analysis. — *Tellus* 62B: 280–295.

Omstedt, A., Edman, M., Claremar, B., Frodin, P., Gustafsson, E., Humborg, C., Hägg, H., Mörth, M., Rutgersson A., Schurgers, G., Smith, B., Wällstedt, T. & Yurova, A. 2012: Future changes in the Baltic Sea acid–base (pH) and oxygen balances. *Tellus* 64B, doi:10.3402/tellusb.v64i0.19586.

Orr, J. C. 2011: Recent and future changes in ocean carbonate chemistry. — In: Gattuso, J. P. & Hansson, L. (eds.), *Ocean acidification*: 41–66. Oxford University Press, New York.

Ortmann, C. & Grieshaber, M. K. 2003: Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. — *Journal of Experimental Biology* 206: 4167–4178.

Parker, L. M., Ross P. M., O'Connor, W. A., Borysko, L., Raftos, D. A. & Pörtner, H. O. 2012: Adult exposure influences offspring response to ocean acidification in oysters. — *Global Change Biology* 18: 82–92.

Pedersen, T. F. 1992: Temporal variations in heat dissipation and oxygen uptake of the soft shell clam *Mya arenaria* L. (Bivalvia). — *Ophelia* 36: 203–216.

Piersma, T., Van Gils, J., De Goeij, P. & Van Der Meer, J. 1995: Holling's functional response model as a tool to link the food-finding mechanism of a probing shorebird with its spatial distribution. — *Journal of Animal Ecology* 64: 493–504.

Pörtner, H. O., Langenbuch, M. & Michaelidis, B. 2004: Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and Earth history. — *Journal of Oceanography* 60: 705–718.

Pörtner, H. O. 1987: Contributions of anaerobic metabolism to pH regulation in animal tissues: theory. — *Journal of Experimental Biology* 131: 69–87.

Pörtner, H. O. 2008: Ecosystem effects of ocean acidification in times of ocean warming: a physiologists view. — *Marine Ecology Progress Series* 373: 203–217.

Reipschläger, A. & Pörtner, H. O. 1996: Metabolic depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus*. — *The Journal of Experimental Biology* 199: 1801–1807.

Reise, K. 1983: Biotic enrichment of intertidal sediments by experimental aggregates of the deposit-feeding bivalve *Macoma balthica*. — *Marine Ecology Progress Series* 12: 229–236.

Ries, J. B., Cohen, A. L. & McCorkle, D. C. 2009: Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. — *Geology* 37: 1131–1134.

Saderne, V., Fietzek, P. & Herman, P. M. J. 2013: Extreme variations of pCO_2 and pH in a macrophyte meadow of the Baltic Sea in summer: evidence of the effect of photosynthesis and local upwelling. — *PLoS ONE* 8: e62689, doi:10.1371/journal.pone.0062689.

Schalkhausser, B., Bock, C., Stemmer, K., Brey, T., Pörtner, H. O. & Lannig, G. 2012: Impact of ocean acidification on escape performance of the king scallop, *Pecten maximus*, from Norway. — *Marine Biology* 160: 1995–2006.

Sokolowski, A., Wolowicz, M., Hummel, H. & Bogaards R. 1999: Physiological responses of *Macoma balthica* to copper pollution in the Baltic. — *Oceanologica Acta* 22: 431–439.

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M. C., Dupont, S. T. 2011a: CO_2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. — *Comparative Biochemistry and Physiology Part A* 160: 331–340.

Stumpp, M., Dupont, S., Thorndyke, M. C. & Melzner, F. 2011b: CO_2 induced seawater acidification impacts sea urchin larval development II: Gene expression patterns in pluteus larvae. — *Comparative Biochemistry and Physiology Part A* 160: 320–330.

Tallqvist, M. 2001: Burrowing behaviour of the Baltic clam *Macoma balthica*: effects of sediment type, hypoxia and predator presence. — *Marine Ecology Progress Series* 212: 183–191.

Taylor, A. C. & Brand, A. R. 1975: A comparative study of the respiratory responses of the bivalves *Arctica islandica* (L.) and *Mytilus edulis* L. to declining oxygen tension. — *Proceedings of the Royal Society B* 190: 443–456.

Thomas, H. & Schneider, B. 1999: The seasonal cycle of carbon dioxide in Baltic Sea surface waters. — *Journal of Marine Systems* 22: 53–67.

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A. & Melzner, F. 2013: Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. — *Global Change Biology* 19: 1017–1027.

Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M. & Melzner, F. 2010: Calcifying invertebrates succeed in a naturally CO_2 enriched coastal habitat but are threatened by high levels of future acidification. — *Biogeosciences* 7: 5119–5156.

Thomsen, J. & Melzner, F. 2010: Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. — *Marine Biology* 157: 2667–2676.

Väinölä, R. 2003: Repeated trans-Arctic invasions in littoral bivalves: molecular zoogeography of the *Macoma balthica* complex. — *Marine Biology* 143: 935–946.

Van Colen, C., Debusschere, E., Braeckman, U., Van Gansbeke, D. & Vincx, M. 2012: The early life history of the clam *Macoma balthica* in a high CO_2 world. — *PLoS ONE* 7: e44655, doi:10.1371/journal.pone.0044655.

Walther, K., Anger, K. & Pörtner, H. O. 2010: Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54 vs. 79 N). — *Marine Ecology Progress Series* 417: 159–170.

Wang, W. X. & Widdows, J. 1993: Calorimetric studies on the energy metabolism of an infaunal bivalve, *Abra tenuis*, under normoxia, hypoxia and anoxia. — *Marine Biology* 116: 73–79.

Whiteley, N. M. 2011: Physiological and ecological responses of crustaceans to ocean acidification. — *Marine Ecology Progress Series* 430: 257–271.

Widdicombe, S. & Spicer, J. I. 2008: Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? — *Journal of Experimental Marine Biology and Ecology* 366: 187–197.

Zwarts, L. & Wanink, J. 1989: Siphon size and burying depth in deposit- and suspension-feeding benthic bivalves. — *Marine Biology* 100: 227–240.