

The effect of carbon dioxide-induced water acidification on physiological processes of Baltic invertebrates

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The atmospheric concentration of carbon dioxide has increased significantly since the pre-industrial times, primarily due to fossil fuel combustion. Oceans have already absorbed approximately half of the total anthropogenic carbon dioxide released into the atmosphere. Dissolved CO₂ readily reacts with seawater to form carbonic acid, which immediately dissociates. This increases the ocean acidity and shifts the distribution of inorganic carbon species towards the increased CO₂ concentrations and decreased concentration of carbonate ions. The increase in hydrogen ion concentration reduces the pH of water. Therefore, since the beginning of the industrial period, pH of oceans has decreased by 0.1 units. It is expected that with the increasing CO₂ levels, pH of the ocean could be reduced by the end of this century by as much as 0.4 units. This phenomenon, referred to as ocean acidification, is considered as one of the main threats to the marine biodiversity. The reason is that CO₂ easily diffuses into body fluids and acidifies both intra and extracellular compartments causing disturbances in the acid-base balance. Furthermore, this has further, severe implications on different physiological processes. Numerous studies have reported that pH, or CO₂ partial pressure values predicted for oceans in the near future negatively affect e.g. behaviour, osmotic concentration, oxygen transport, the metabolic rate, calcification, protein synthesis, the growth, reproduction and in turn, survival of marine organisms. Consequently, it may have serious implications on the ecosystem level. Locally, in highly productive coastal waters, the effect of ocean acidification could also be amplified by another anthropogenic problem - the eutrophication. Although a similar trend of surface pH reduction, like in the oceanic waters, is observed in the Baltic Sea, decomposition of excessive amounts of organic matter may additionally intensify the CO₂ production leading to an increase in acidity, especially near the bottom layers. It is calculated that some coastal areas of the Baltic Sea are already characterized by pH values that are much lower than what is predicted for the open ocean surface waters by the end of this century. Similarly to other coastal regions of this reservoir, the Gulf of Gdańsk is also characterized by significant seasonal pH fluctuations greater than one pH unit in the surface and bottom layers, which is connected with the riverine nutrient input, mineralization and the water dynamics [1]. Consequently, organisms that inhabit coastal areas of the Baltic Sea, especially benthic fauna, are exposed to rapid and

severe changes in pH and carbonate chemistry which potentially adds more stress which organisms have to cope with. However, future effects of ocean acidification, superimposed on such a dynamic system, on carbonate chemistry, as well as its physiological consequences are much more difficult to predict compared to surface oceanic waters. It is also worth mentioning that most of the coastal ecosystems are dynamic environments where acidification could co-occur in combination with multiple, different stressors. The interactions between acidification and other adverse environmental factors are often complex and they might act synergistically. In the Baltic Sea, the increasing eutrophication process, which leads to the pH fluctuations, is also the main cause of oxygen deficiency – an important stressor that negatively affects the behaviour and physiology of benthic fauna. Despite the above facts, the available data on the effects of acidification on the Baltic organisms are very scanty. They are limited to a few studies on the acid-base status, respiration, growth and gene expression of two bivalve species, mainly from the western Baltic Sea. Research concerning the potential synergistic effect of acidification and other factors on Baltic organisms are completely missing. It is also worth mentioning that while studies concerning the influence of acidification on different marine taxa have come increasingly into focus in recent years, only few are related to behavioural and metabolic changes, which are the first noticeable responses of an organism exposed to environmental stress and non-specific indicators of environmental stress. It should also be kept in mind that responses to acidification are highly species-specific and can vary significantly even between closely related taxa. Also intraspecific variability connected with some local adaptations is often observed. Therefore, there is an urgent need for further research, especially concerning organisms from naturally CO₂-enriched sites.

Given the above, the overall objective of the presented dissertation was to determine the effect of carbon dioxide-induced water acidification on physiological processes of four invertebrate species, which are important components of the Baltic benthic communities, an isopod *Saduria entomon*, an amphipod *Gammarus oceanicus*, Baltic clam *Macoma balthica* and blue mussel *Mytilus edulis trossulus*, representing crustaceans and bivalves. To this end, changes in the metabolic rate, reflecting changes in all physicochemical processes taking place in an organism, were studied under short- (12 hours) or long-term (14 days) and under exposure to different pH treatments (8.2-6.0) [1-4]. For this purpose, the direct calorimetry based on heat dissipation measurements was applied, as it is the only direct and non-invasive method that allows to measure the total metabolic rate of an organism. It is especially important in the studies of organisms that are able to use anaerobic pathways to produce

energy, like some invertebrates, where indirect methods (e.g. respiration) do not provide reliable information. Moreover, it is also essential to keep in mind that the first noticeable responses of an organism exposed to stress are changes in its behaviour. Although, the metabolic rate is one of the common physiological variables used as a non-specific indicator of animal performance and adaptation under environmental stress, it is closely dependent on the organism's behaviour. Thus, it is important to monitor the metabolism and behaviour simultaneously so as to distinguish the resting and active levels and to effectively determine the effect of the studied factor. Direct calorimetry is the tool that enables continuous registration of both parameters. Therefore, it was also aimed to examine changes in the behaviour (locomotor activity in crustaceans and shell opening and closing in bivalves) in all species [1-4]. In the isopod *S. entomon* [1] and the Baltic clam *M. balthica* [3], another behavioural parameter, used in the infaunal species as an indicator of stress conditions – the burrowing activity, was evaluated [1,3]. Additionally, the hemolymph osmolality [1,2] as well as the concentration of the chloride ion and hemolymph pH [1] were evaluated in *S. entomon* and in the amphipod *G. oceanicus*. Hemolymph osmolality or osmotic capacity (the difference between osmotic pressure of the hemolymph and external medium) are a reliable indicators of environmental stress in crustaceans, especially when connected with ionic changes in the water. Monitoring of this parameter is extremely important in species of marine origin inhabiting the Baltic Sea, due to the fact that they maintain hemolymph osmolality at a level much higher than the surrounding, hypoosmotic medium and thus need additional energy for the osmotic adjustment. In the case of the blue mussel *M. edulis trossulus*, the objective was also to determine whether the effect of water acidification on the metabolic rate and shell gaping behaviour is additionally amplified by low oxygen saturation, because those factors commonly co-occur in the environment [4].

It was assumed that acidification will affect the behaviour of the studied species, which in the case of crustaceans might be connected with the increased locomotor activity as an attempt to escape from unfavourable environment. On the other hand, acidification in bivalves may trigger the responses to isolate from adverse conditions and to close shell valves, which may reduce the activity and in turn, the metabolic rate. Also the behaviour of *S. entomon* and *M. balthica* connected with burrowing in the sediment may be altered by low pH, according to the fact that infaunal species usually reduce their burial depth and move to the sediment surface during long-term, unfavourable conditions. It is connected with worse conditions in the sediment compared to the overlying water. During the acidification of body fluids, the process of ionoregulation represents the only attempt to restore the acid-base

balance. Lack of the compensation of body fluids' acidosis usually causes reduction in the metabolic rate. Thus the metabolic suppression connected not only with the decreased activity, but also as a result of uncompensated acidosis of body fluids might be observed in bivalves, as they have poorly developed ion-exchange mechanisms. On the other hand, crustaceans may have rather elevated metabolic rates as a result of upregulated branchial $\text{Na}^+/\text{K}^+/\text{-ATPase}$ and other enzymes which usually considerably increase the energy expenditures. However, this might be coupled with some changes in hemolymph osmotic concentration. It was also hypothesized that simultaneous exposure to low pH and oxygen saturation would affect (more than a single factor) the natural behaviour of *M. edulis trossulus* by triggering the responses to isolate from adverse conditions. This also might significantly suppress its metabolic rate because both factors have been suggested to elicit the metabolic depression by altering the acid-base status of body fluids. It might also be assumed that bivalves (considered poor iono- and osmoregulators) will be generally more affected by acidification than crustaceans which usually have well-developed ion-exchange mechanisms. On the other hand, the effect of acidification on the Baltic invertebrates might not be very severe compared to organisms from other regions. Individuals living in the temporarily acidified environment of the Baltic Sea might be to some extent resistant and adapted to cope with environmental hypercapnia. In the studied species, some adaptations to other adverse factors (e.g. hypoxia, low salinity, eutrophication) might also induce the development of some pre-adaptations to acidification.

The obtained results indicated that the short-term exposure to carbon dioxide-induced water acidification did not affect behaviour of the studied crustaceans and bivalves, as they behaved naturally. They exhibited periods of rest and activity (peaks) indicated by low or high values of heat production respectively. It was observed that the active metabolic rate as well as the ratio between the active and resting rate (scope for activity) remained unchanged despite the changes in water pH [1-4]. Moreover, there were no changes in gaping activity (shell opening and closing) of *M. balthica* and *M. edulis trossulus* during exposure to different pH treatments [3,4]. The burrowing activity, measured after long-term exposure also confirmed the natural, unaffected by low pH behaviour of *S. entomon*, which usually spends most of its life buried in the sediment [1]. The burrowing behaviour of *M. balthica* was significantly different in the acidified variants compared to the control treatment. However, the high percentage of individuals observed on the sediment surface in lowest pH may suggest that the Baltic clam does not need to greatly decrease its activity and preserve the energy while feeding, which may also be considered an adaptation to the studied factor [3].

Crawling behaviour connected with the deposit feeding demands more energy than suspension feeding by a siphon during burial.

The presented study provides evidence that short-term exposure to water acidification did not significantly affect the resting metabolic rate of any of the studied species [1-4]. The obtained results suggest that acidification did not induce the metabolic depression or increase the energy demand. This should be considered very beneficial for the studied organisms, because both the metabolic depression and the metabolic increase result in trade-offs in energy allocation between different processes. The ability of the studied species to remain active, and probably aerobic in the hypercapnic conditions should be considered as an adaptation that enables them to sustain basic physiological functions such as, for example, feeding and ventilation. In the blue mussel *M. edulis trossulus*, the synergistic effect of decreased pH and oxygen saturation on the behaviour and metabolic rate was not observed either. Contrary to pH, however, low oxygen saturation was a signal to reduce the activity and to isolate from adverse conditions which was reflected in a significant decrease in time spent active. This indicates that hypoxia is a more stressful factor for this species than acidification [4]. On the other hand, it might be supposed that adaptations of Baltic bivalves to periodic hypoxia may have also contributed to the increased tolerance to acidification. During the shell closure, the limited gas exchange with surroundings, as well as metabolic acids produced during anaerobiosis result in respiratory acidosis of body fluids. However, species that are able to cope with hypoxia developed some adaptations to long term survival such as a decreased amount of protons and a decreased rate of their accumulation.

Nonetheless, a different situation was observed in *M. balthica* after the long-term exposure to the reduced pH. Both, the resting and active metabolic rate increased in the lowest pH treatment (6.0) in contrast to the control conditions. This may be related to an additional cost of acid-base regulation and the expression of biomineralization-related enzymes. This should be considered an adaptation to the studied factor, especially that as it is known from literature, the elevated metabolic rate is one of the key processes involved in the adaptation to elevated pCO₂. However, the scope for activity was slightly reduced at the lowest pH indicating the allocation of energy from activity to e.g. acid-base regulation, which can be disadvantageous over a longer period of time [3]. The ability of the studied bivalves to maintain unchanged or even increased metabolic rates during acidification might be connected with the fact that *M. edulis trossulus* as well as *M. balthica* may also benefit from eutrophication. The elevated nutrient concentrations leading to increase in algal blooms in the Baltic Sea enable bivalves to obtain the sufficient energy for growth, to sustain high

metabolic rates and therefore to cope with environmental stress, presumably including acidification.

The obtained results indicate that Baltic crustaceans are able to maintain high hemolymph osmolality after both short- and long-term exposure to acidification, which was observed in *G. oceanicus* and *S. entomon*, respectively [1,2]. High osmolality maintained by *G. oceanicus* during hypercapnia did not generate additional physiological costs as the metabolic rate remained unchanged in all pH treatments [2]. Although the metabolic rate in *S. entomon* did not change after the exposure to low pH, the measurements were made after a shorter period than osmoregulation. Nonetheless, high hemolymph osmotic concentration in this species was associated with hemolymph alkalinity in hypercapnic treatments, which indicates very effective compensation of the pH reduction. Surprisingly, no changes were observed in the chloride ion concentration in the hemolymph of *S. entomon*, which means that this species uses a different effective mechanism for regulating the acid-base imbalance than the exchange of chloride ions for bicarbonates, often observed in crustaceans [1]. The fact that the maintenance of high hemolymph osmolality was probably not energetically expensive might indicate that the studied species are preadapted to large fluctuations in CO₂ levels observed in the environment. On the other hand, it might be presumed that the studied crustacean species during long-term adaptation to living in the hypoosmotic environment in the Baltic Sea developed mechanisms which also make them preadapted to water acidification, because they are very similar. These hyperregulating crustaceans already required additional energy for osmotic adjustment in hyposaline environment, thus the above mechanisms might already operate at the full energetic cost. The above-mentioned adaptations of the studied species were also reflected in their high survival during long-term exposure to acidification [1,3].

The study confirmed the assumption that Baltic invertebrates are generally resistant to CO₂-driven water acidification. However, the studied species proved to be even more resistant and adapted to this factor than it was expected, since most of them did not show any changes in the investigated parameters. Also the observed changes should be considered an adaptation to the studied factor. Moreover, the studied bivalves proved to be as resistant to acidification as crustaceans. Moreover, this resistance has not been amplified even by low oxygen saturation. The current thesis provides, for the first time, the information concerning the effect of CO₂-driven low pH on invertebrates from the southern Baltic Sea and in general the first information about the effect of this stressor on Baltic crustaceans. Also the effect of pH and low oxygen saturation, acting simultaneously on the Baltic organisms, was examined

for the first time. The thesis presents also the first studies in which behaviour and metabolic rate under acidification were determined using the direct calorimetry. Moreover, the obtained results significantly complement the information about the effect of acidification on the osmoregulation in a marine organism, which is limited, especially regarding the potential cost of osmotic adjustment during the hypercapnic exposure. Therefore, it seems that the presented dissertation represents an important contribution to research concerning ecophysiology of marine organisms and therefore significantly supply the world literature.

References:

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