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Impact of potential CO_2 leakage from sub-seabed storage site in the southern Baltic Sea on the Baltic clam *Limecola balthica* under relevant hydrostatic pressure

With ongoing increase of carbon dioxide emission to the atmosphere Carbon Capture and Storage technology (CCS) in sub-seabed geological reservoirs receives growing attention as an important solution for reduction of CO_2 emissions for climate change mitigation. Its implementation in the southern part of the Baltic Sea has been a subject of scientific and administrative discussions over recent years. Potential CO_2 storage sites include an oil- and gas-carrying B3 field which is currently operated by Grupa Lotos S.A. in the Polish Exclusive Economic Zone. Large capacity (several million tons) and firm geological structure of the cap-rock make the B3 field a good candidate for offshore geological storage of carbon dioxide. Though the CCS is conceived as safe technology, it still poses potential environmental hazards of which CO_2 leaking is considered of the greatest marine environmental risk. Studies on ecological risk assessment in case of CO_2 leakage from sub-seabed site have been conducted worldwide but few addressed the southern Baltic Sea which shows specific environmental characteristics. CO_2-driven alterations in overlying bottom and interstitial waters can become an additional threat to resident benthic fauna which is exposed to low salinity, eutrophication and occasionally anoxic conditions. Due to increased solubility of gaseous carbon dioxide at elevated hydrostatic pressure and low salinity, decreased water pH caused by potential CO_2 leak might have specific biological impacts in brackish areas. Therefore, in this study, the effect of CO_2-induced seawater acidification on the key benthic species, the Baltic clam *Limecola balthica* (Linnaeus 1758) from the southern Baltic Sea was investigated in the laboratory under relevant hydrostatic pressure. Surface sediments and clams were collected in April 2016, October 2016 and January 2017 at one coastal site (MW) in the Gulf of Gdańsk where environmental conditions in the bottom zone are similar to those in the B3 field. Experimental seawater pH range: 7.7 - control, 7.0 and 6.3 was selected to mimic changes in the overlying bottom water acidity following CO_2 leakage from the sub-seabed storage site. Three independent experiments were run for 50 days, each under relevant hydrostatic pressure of 900 kPa (80 m water depth) using a unique titanium flow-through system - the Karl Erik hyperbaric tank (TiTank, NTNU, Trondheim). In each experiment, sediments and animals were acclimatized to experimental conditions for 10 days and then exposed to the target seawater pH

over the next 40 days. During all experiments basic water parameters (salinity, temperature, oxygen saturation, redox, pH and hydrostatic pressure) were monitored and the animals were fed with cultured unicellular algae suspension at a constant rate. In the Baltic clam from the southern Baltic Sea the selected biological markers of environmental hypercapnia showed differing responses. Prolonged seawater acidification did not exert lethal effects on the Baltic clam, overall survival rate (ca. 90%) was high in all experiments. Modest changes in morphometric condition indices, little utilization of high-energy reserves (lipids, carbohydrate, proteins) and slight shell growth variation suggest activation of effective pH compensatory mechanisms. Additional energy requirements for maintenance of acid-based balance in the tissues were covered likely from assimilated food. Unlimited food availability and increased hydrostatic pressure have been hypothesized to affect shell growth of the bivalves under acidic conditions. Seawater pH 7.0, induced deeper burrowing of the clams in sediment than pH 7.7 and 6.3 conditions that might indicate protective behavioral response to increased and prolonged surface sediment toxicity. Response patterns of oxygen-dependent enzymes associated with energetic metabolism, i.e. high overall malate dehydrogenase (MDH) activity and occasional up- and downregulation of lactate dehydrogenase (LDH) and octopine dehydrogenase (ODH) indicated high metabolic plasticity of the clams under acidic conditions. Out of the antioxidant enzymes investigated: catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione peroxidase (GPx), inhibited activity was observed only for GPx at moderate hypercapnia. Downregulation of glutathione peroxidase indicated induction of temporal cellular stress that was manifested also by enhanced concentration of oxidative stress products (lipid and protein peroxidation products) in the soft tissues of the clams. Upregulation of acetylcholinesterase (AChE) activity in the clams kept at pH 7.0 suggests an increase of ambient toxicity. The results obtained in this study support further the hypothesis of high resilience of the Baltic clam to seawater acidification, especially to extreme ambient seawater acidity (pH 6.3). The Baltic clam *Limecola balthica* is able to withstand acidic conditions owing to its physiological plasticity and avoidance strategy. Low sensitivity of the Baltic clam to reduced seawater pH stems presumably from pre-adaptation to natural environmental pH fluctuations that occur in organic-rich stratified sediments of the southern Baltic Sea. The study delivered important findings on the biological response of macrobenthic infauna to potential consequences of CO_2-injection under the seabed and thus supported environmental risk assessment in terms of CCS implementation in the Southern Baltic Sea.