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Differing responses of the estuarine bivalve *Limecola balthica* to lowered water pH caused by potential CO_2 leaks from a sub-seabed storage site in the Baltic Sea: An experimental study

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ABSTRACT

Sub-Seabed CCS is regarded as a key technology for the reduction of CO_2 emissions, but little is known about the mechanisms through which leakages from storage sites impact benthic species. In this study, the biological responses of the infaunal bivalve *Limecola balthica* to CO_2-induced seawater acidification (pH 7.7, 7.0, and 6.3) were quantified in 56-day mesocosm experiments. Increased water acidity caused changes in behavioral and physiological traits, but even the most acidic conditions did not prove to be fatal. In response to hypercapnia, the bivalves approached the sediment surface and increased respiration rates. Lower seawater pH reduced shell weight and growth, while it simultaneously increased soft tissue weight; this places *L. balthica* in a somewhat unique position among marine invertebrates.

1. Introduction

Carbon Capture and Storage (CCS) is regarded a key mitigation technology for the reduction of CO_2 emissions from fossil fuel power stations and other industrial sources (IPCC, 2005). CCS involves capturing waste CO_2 from point emission sources and depositing it in deep geological formations such as oil and gas fields and unmineable coal seams (Widdicombe et al., 2013a) to prevent it from entering the atmosphere. The technology has also been implemented in the marine environment where CO_2 is injected into deep sub-seabed formations such as deep oil and gas reservoirs or aquifers. In Europe, two storage sites have been operated on a commercial scale by STATOIL for more than ten years, i.e., the Sleipner field in the North Sea and the Snøhvit field in the Barents Sea on the Norwegian Continental shelf (Riis and Halland, 2014). While CCS technology could potentially reduce CO_2 emissions by as much as 80–90% (Holloway, 2007), storing large volumes of liquid CO_2 raises concerns about the environmental consequences of potential leakage (Blackford et al., 2009). Although it is generally accepted that leakage will occur over time (Hawkins, 2004; de Orte et al., 2014; Clements and Hunt, 2017), the risks associated with CO_2 seepage, its form, and the spatial extent of consequential seawater acidification remain largely unknown (Koornneef et al., 2010; Lichtschlag et al., 2015; Taylor et al., 2015). Thus, the nature of CO_2 release, its temporal evolution, and its impact on geochemical processes and benthic and pelagic fauna have become urgent research topics since they provide important support for CSS environmental risk assessments.

In recent years, a potential CO_2 storage site has been proposed in an abandoned oil reservoir (B3 field) at a water depth of 80 m and a depth under the seabed of 1450 m in the Polish sector of the southern Baltic Sea (the Polish Exclusive Economic Zone). The CO_2 stored in the B3 field would include compressed refinery generated CO_2 (ca. 450 kt y^{-1}) and liquid CO_2 from the proposed integrated gasification combined cycle (IGCC) power plant (1 Mt y^{-1}). Additionally, after the conclusion of the exploitation of natural gas fields B4 and B6, which are operated by LOTOS Petrobaltic S.A., geological storage of CO_2 will be considered at these two prospective offshore sites. Both of these oil and gas fields are located in a tectonically stable area, and they are covered with Silurian deposits of thicknesses ranging from 1000 to 2000 m, which is assumed to prevent CO_2 leakage (Zajfert et al., 2015).

One major environmental consequence of CO_2 leakage from sub-seabed storage is localized reductions in seawater pH (acidification), particularly in the overlying bottom zone (Chen et al., 2005), which directly impacts resident benthic organisms inhabiting surface sediments (Kroeker et al., 2013). Although recent advancements in controlled laboratory experiments (e.g., Lee et al., 2016), exposure experiments on organisms in situ (e.g., Ishida et al., 2013), and studies at

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analogue sites (natural CO_2 seeps; e.g., Fabricius et al., 2014) have improved considerably our understanding of the physiological and ecological responses to increased CO_2 concentrations, the general mechanisms through which leakages could impact marine species are not necessarily uniform across different species or environmental variables (Walther et al., 2010). For example, different magnitudes and rates of the effects of high CO_2 levels have been observed in infaunal and epifaunal macroinvertebrates that are exposed naturally to different pH levels in the ambient environment (Widdicombe et al., 2013b). Divergent patterns of compensative/defensive biological traits and physiological performance can be also expected in areas where benthic organisms are adapted/acclimated to specific local conditions (e.g., low salinity, large nutrient and organic matter loads, oxygen deficits) such as the Baltic Sea.

Since the last glaciation 8000 years ago, the young Baltic ecosystem has been undergoing continuous post-glacial successional changes that are driven by strong physical and chemical environmental gradients (e.g., salinity and carbon) and ecological diversity (Bonsdorff, 2006). Together with large freshwater inputs and high anthropogenic pressure (including eutrophication and pollution), this creates harsh ecological conditions in the Baltic, particularly in the bottom zone, including high rates of organic matter sedimentation, low dissolved oxygen concentrations, and the formation of hydrogen sulphide (Meier et al., 2012). As a result, most deep-water benthic communities are decimated and species poor, comprising taxa that are able to withstand prolonged periods of hypoxia and anoxia and the temporal presence of H_2S in the interstitial and overlying bottom waters (Gogina et al., 2016) such as the Baltic clam *L. balthica*. In addition, the reduced alkalinity and low salinity of the Baltic waters decrease buffering capacity toward CO_2 that ultimately results in higher seawater pH fluctuations than in the open ocean. Therefore, caution should be exercised when inferences about the likely effects of seawater acidification on Baltic benthic species are drawn based on results from other coastal areas (Havenhand, 2012). Investigations of the potential impact of increased seawater CO_2 concentration on marine adult infaunal bivalves inhabiting the organic-rich soft sediments of the Baltic Sea are scarce (e.g., Havenhand, 2012; Jansson et al., 2013, 2016; Jakubowska and Normant-Saremba, 2015) and lag behind those performed in fully saline environments of similar latitude (for review see Ries et al., 2009; Kroeker et al., 2013; Clements and Hunt, 2017). There is an urgent need for more controlled mesocosm experiments on organisms inhabiting this water basin, particularly benthic species that dominate the sea floor and constitute key elements of trophic links and ecosystem functioning (Havenhand, 2012).

The aim of the study was to determine and quantify the impact of CO_2-induced seawater acidification on the Baltic clam *L. balthica* from the southern Baltic Sea (Gulf of Gdańsk) using mesocosm experiments. By setting experimental conditions within a broad range of seawater pH 7.7–6.3, which simulates potential scenarios of changes in acidity of the overlying bottom water if there is carbon dioxide leakage from the sub-seabed storage site, the biological response of this bivalve species was investigated to support environmental risk assessments of CCS implementation in the southern Baltic Sea.

2. Material and methods

2.1. Model species

The Baltic clam *L. balthica* is an infaunal tellinid bivalve commonly present in marine and estuarine soft-sediment habitats along both coasts of the North Atlantic. In Europe, it occurs from the White Sea (70°N) in the north to the Charente Estuary (45°N) in the south where it usually inhabits tidal and subtidal, sandy and muddy bottoms to a maximal depth of 30 m (Jansen et al., 2007). The brackish Baltic Sea is the only water basin where the clam can appear from a shallow sublittoral non-tidal zone to a deep-water aphotic zone, even down to 191 m (Jansson et al., 2013 and citations therein). In sandy and muddy substrates, the optimal burrowing depth of the clam, > 10 mm shell length, ranges from 20 mm in summer to 50 mm in winter (Zwarts and Wanink, 1989). In the Baltic soft sediments, *L. balthica* can be found in large numbers both in inner semi-enclosed basins, e.g., the Gulf of Gdańsk (up to 990 ind. m^{-2}; Sokołowski, 2009), and in deeper open waters including the B3 field (several ind. m^{-2}; ECO2, 2014), often dominating the biomass of benthic communities and playing a key role in sediment reworking and bioturbation (Jansson et al., 2013). The bivalve is even able to live in seriously polluted regions where other benthic species that are considered to be resistant are reduced in number or absent. The wide distribution across a range of environmental qualities implies the likely presence of specific adaptations or genetic differentiation of *L. balthica* and its broad tolerance of environmental variables (Sokołowski et al., 2004). On the other hand, it has been shown that bivalve physiological performance and adaptive capacity to additional stressors, such as elevated temperature, decrease in continuously or temporally altered environments. Higher stress sensitivity presumably stems from the poor energetic situation of organisms that must increase metabolic activity to deal with adverse conditions (Hummel et al., 1997).

2.2. Collection and pre-treatment of sediment and bivalves

Sediment and bivalves were collected at one coastal site (MW; φ 54°37′30.6″N λ 18°37′25.8″E) in the Gulf of Gdańsk (southern Baltic Sea), at a water depth of 30 m on April 3, 2014. The location of the sampling site was selected to represent geochemical and ecological conditions similar to those in the area of the potential sub-seabed CO_2 storage site (oil-carrying B3 field currently exploited by LOTOS Group S.A.) in the Polish sector of the southern Baltic Sea. Dissolved oxygen concentration in the overlying bottom water in the vicinity of the MW site varies seasonally reaching > 10.0 mg dm^{-3} in cold seasons with occasional deficits and the temporal presence of hydrogen sulphide during warm stagnant periods (Janas et al., 2004). Water temperature generally reflects local meteorological conditions with higher values in summer (up to 21.0 °C) and lower values in winter (down to 1.6 °C), and salinity that ranges between 6.8 and 7.2 (Sokołowski, 2009). An inventory field campaign in the B3 field in September 2012 showed that oxygen conditions above the seafloor ranged from 0.5 mg dm^{-3} to 5.6 mg dm^{-3} (mean ± SE; 3.3 ± 0.3 mg O_2 dm^{-3}), the temperature was between 6.1 and 10.3 °C (7.8 ± 0.3 °C), and salinity varied from 8.9 to 10.2. At most sites the concentration of hydrogen sulphide did not exceed 0.1 μmol dm^{-3} (ECO2, 2014). Surface sediment was sampled with a Van Veen grab (0.1 m^2 catch surface area) and the upper 10-cm layer was collected with a spatula. The sediment was then stirred and sieved through a 1.0-mm mesh to remove macrofauna (e.g., priapulids, crustaceans), large particles, and debris, and then placed in a transport container and covered with seawater to prevent desiccation and reduce temperature change. The clams were collected by dredging and transported immediately to laboratory in aerated seawater taken in situ. Special care was taken to select individuals of a limited size range (11.0–15.0 mm corresponds to individuals 3–4 years old, which is the dominant size class of the local population; Sokołowski, 1999; Jansen et al., 2007) as size has been demonstrated to sometimes affect the biological responses of bivalves (Sukhotin et al., 2003). Additionally, samples of overlying bottom seawater (ca. 0.2 m above the sea floor) were taken with a 5-dm^3 GoFlo Niskin water sampler to record basic hydrological parameters (salinity, temperature, pH, dissolved O_2) with a portable WTW Multiset 340i meter.

2.3. Experimental set up and sampling protocol

In the mesocosm, bivalves were acclimated to experimental conditions. Water temperature was increased from 4.3 °C (ambient overlying bottom seawater) to 10.5 °C at a rate of 1 °C 24 h^{-1} over six days