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Effects of experimental long-term CO_2 exposure on *Daphnia magna* (Straus 1820): From physiological effects to ecological consequences

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HIGHLIGHTS

- *Daphnia magna* was used in a CO_2 injection experiment, simulating a CCS leak scenario.
- Survival, individual growth, RNA:DNA ratio, and neonates production were analysed.
- Secondary production effects were detected, highlighting the ecological implications.

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ABSTRACT

The carbon capture and storage (CCS) technologies that were proposed to mitigate environmental problems arising from anthropogenic CO_2 emissions, also have potential environmental risks. An eventual CCS leak might induce very low pH values in the aquatic system. Due to the lack of knowledge of long-term CO_2 exposures with very low pH values, this study aims to know the effects and consequences of such a situation for zooplankton, using the *Daphnia magna* experimental model. A CO_2 injection system was used to provide the experimental condition. A twenty-one days experiment with control and low pH treatment (pH = 7) replicates was carried out under light and temperature-controlled conditions. Survival, individual growth, RNA:DNA ratio, and neonates production were analysed during the aforementioned period. No differences on survival (except last day), individual growth and RNA:DNA ratio were observed between both control and low pH treatments. However, clear differences were detected in neonates production and, consequently, in population growth rates and secondary production. The observed differences could be related with an energy allocation strategy to ensure individual survival but would have ecological consequences affecting higher trophic levels.

1. Introduction

New technologies that capture CO_2 have been proposed to mitigate environmental problems arising from man-made CO_2 emissions (Halsband and Kurihara, 2013a,b). The IPCC (2005) estimates that carbon capture and storage (CCS) technologies could have an economic potential between 10% and 55% of the total carbon mitigation strategies by the end of the century. These technologies pose their own risks for instance, an eventual CCS leak, where very low pH values might be reached. So a prerequisite, more knowledge is required to complete risk assessments on CCS. The recent and vast bibliography concerning potential effects of ocean acidification (OA) is based on a future scenario in which the average surface seawater pH ranges from 8.2 to 7.6 by year 2100 (Caldera and Wickett, 2003; IPCC, 2007), and is also based on short-term CO_2 experimental exposures. Nevertheless, some authors suggest pH values lower than 6, near to the point of leakages (Herzog et al., 1996). The environmental risk assessment must be based primarily on laboratory and small-scale experiments, because such large amounts of relatively pure CO_2 have never been introduced into the deep ocean in a controlled experiment (IPCC, 2005). However, since most of the current information came from short-term CO_2 experimental exposures, it is necessary to invest more effort in decreasing the gap between these predominant short-term experiments and the long-term exposure

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that might take place near to a CCS leak. This applies especially to those organisms, such as zooplankton, that have a limited ability to move across water masses (Herzog et al., 1996) even though a recent study has reported higher zooplankton capacity to respond under moderate levels of turbulence (Michalec et al., 2015).

The present research is based on laboratory experiments, but with the intent of understanding the effects of CO_2 leakages in natural populations, as was recommended by Gómez et al. (2001), who argued that laboratory investigations with model zooplankters are needed in order to improve field measurements. The main shortcoming of those experimental tests is the lack of ecological relevance, because the ecological interactions are ignored and solely standard species are used, instead of local species. However, they are necessary as a first step to achieving the initial information about long-term effects. In this sense, Raimondo and Mckenney (2006) state the importance of reproduction in population-level risk assessment and the need for complete life-cycle test data to make an explicit link between the organism and higher hierarchical levels. For instance, secondary production research has been claimed, because it allows to know how material and energy are transferred, and could be used to detect the effects of perturbations on the ecosystem (Jiménez-Melero et al., 2013). Ecologically important endpoints, such as those related to reproduction, have been previously used (Calow et al., 1997; Van Straalen and Kammenga, 1998). Some of them have been proposed to be used as references to calculate the safety factors needed in the Environmental Risk Assessment (ERA) procedures (Roex et al., 2000). The intrinsic rate of population increase r is regarded as being one of the most ecologically relevant, and as a standardised parameter could be used to compare toxic effects (Calow et al., 1997; Forbes and Calow, 2002), and is a more relevant measurement of toxicant effects than traditional measures of mortality or reproduction (Barata et al., 2012). The reduction of population growth rate of a key species could induce significant ecological effects on the rest of a lake community (Hanazato and Dodson, 1995). Although realistic effects at population level are difficult to determine and require long-term observation, the step in seeking the effect on r might be taken. Multi-generation incubations to detect selection pressure of acidification on life cycle traits and adaptation would be desirable (Halsband and Kurihara, 2013a,b) similarly to those described by Souissi et al. (2014) where a generational selection occurred after just five generations under a climate change scenario. From a hierarchical point of view, a link between physiological effects and the population and community consequences is needed to understand the mode of action of toxicants and/or changeable environmental factors. For this reason, biomarkers are being used, increasingly, to bridge the aforementioned gap (De Coen et al., 2000). A biochemical endpoint, such as the RNA:DNA ratio, has also been proposed to achieve the effects of, for instance, food quality or toxic substances on somatic growth and secondary production (Gusmão and McKinnon, 2009; Vrede et al., 2002). In adult zooplankters, RNA:DNA ratios have been, generally, positively correlated with growth rates and egg production (EP) (Wagner et al., 1998; Gorokhova, 2003; Speekmann et al., 2007). This biomarker could be used to assess the effect of water acidification from a physiological point of view.

Considering zooplankton as the link between primary producers and higher trophic levels, the aim of this paper is to understand the effects and consequences of a long-term CO_2 exposure that simulates eventual CCS technology leakages. In order to achieve this goal, *Daphnia magna* (Cladocera: Crustacea − Straus, 1820), was used as the experimental model system. Owing to its small size, but which is big among the zooplankters, its short life cycle, ubiquity and capacity for parthenogenetic reproduction, *D. magna* has been widely-used as an experimental animal in aquatic environmental toxicity testing, and has been adopted by different environmental agencies (US-EPA, 2002; ASTM, 1988; OECD, 1984, 1998). The effects at individual levels and ecological consequences were analysed using survival, individual length, RNA:DNA ratio and egg production as endpoints.

2. Materials and methods

2.1. Experimental conditions

The experimental model chosen, *D. magna*, has a short life cycle, the lasting of the experiment (21 days) has been considered as long-term experimental period. A monoclonal population of *D. magna* was raised from a second-generation neonate (Neonate n. 2) in a natural wetland Laguna Grande (Baeza, Jaén, Spain). The individuals were reared in mineral water plus a mix diet of *Scenedesmus obliquus* (Turpin) Kützing 1833 (Chemical Engineering Laboratory, University of Jaén) and *Cryptomonas pyrenoidifera* Geitler 1922 (Water Institute, University of Granada) as food in a relation of at least, 1.5×10^6 algae cell/individual. The aforementioned algae were routinely maintained in 3N-BBM + V culture medium pH 8.3–8.5 (modified from CCAP, Scotland) under conditions of 20 °C and supplied with a cycle of 12 h light:12 h dark.

The experiments were designed to test the effects on the organisms of CO_2 injection into the water, drawing on the subsequent acidification using the system-simulator, as described in detail by Basallote et al. (2012), De Orte et al. (2014), and Rodríguez-Romero et al. (2014). Aqua Medic AT Control System (Europe) was used to control and maintain the pH in each vessel where the pH electrodes were placed. Before use, pH electrodes of the CO_2-injection system were calibrated and the values obtained throughout the tests were regularly verified by a portable pH-meter (Crison GLP 22). A solenoid valve allowed the adjusting of the pH values when it was detected that the pH had increased by 0.01 units or more; then, CO_2 gas bubbles were injected into each vessel until the required pH value was reached. A computer connected to the AT control system allowed modification of the pH values, as required. In this experiment, all exposure tests were carried out in a temperature- and light-controlled chamber (20 ± 1 °C and a photoperiod of 12:12 h light:dark). Twenty neonates, with no more than 24 h, were placed in each 2 L vessel. Four replicates for control and for low-pH treatments (pH = 7) were used during 21 days of experimental period. Both *S. obliquus* and *C. pyrenoidifera* (mixed) were used as food during the experimental period. The microalgae density, to ensure no food limitation conditions (at least 1.5×10^6 cell/daphnia/day; Díaz-Baez et al., 2004) on each vessel, was monitored daily during the experimental period. Cell density was calculated using the chlorophyll fluorescence measurement (Aquafluor Turner Designs) in each vessel. The following correlations between fluorescence (X) and abundance (Y) were calculated and used for *C. pyrenoidifera* and *S. obliquus*, respectively

$$y = 14.912X - 1283.2 \left(R^2 = 0.99\right)$$

and

$$y = 61.543X + 55494 \left(R^2 = 0.94\right)$$

Water samples, each of 50 mL, were used for the alkalinity analysis (automatic titrator, 848 Titrino Plus devise). Dissolved oxygen and conductivity data were obtained using a multiparametric probe (YSI-556 MPS).

Measured responses included RNA:DNA ratio, survival, adult and embryo size, and reproduction parameters.