Effects of experimental long-term CO₂ exposure on *Daphnia magna* (Straus 1820): From physiological effects to ecological consequences

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**Highlights**

- *Daphnia magna* was used in a CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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.

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1. Introduction

New technologies that capture CO₂ have been proposed to mitigate environmental problems arising from man-made CO₂ 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 CO₂ 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 CO₂ 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 CO₂ experimental exposures, it is necessary to invest more effort in decreasing the gap between these predominant short-term experiments and the long-term exposure...
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 CO₂ 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 (Jimenez-Meleo 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 change 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 (Gusmao 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 CO₂ 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 × 10¹⁰ 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 CO₂ 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 CO₂-injection system were calibrated and the values obtained throughout the tests were regularly verified by a portable pH-meter (Crisón GLP 22). A sole-noid valve allowed the adjusting of the pH values when it was detected that the pH had increased by 0.01 units or more; then, CO₂ 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 × 10¹⁰ 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 (Aqualfluo 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

\[ y = 14.912X - 1283.2 \quad (R^2 = 0.99) \]

and

\[ y = 61.543X + 55494 \quad (R^2 = 0.94) \]

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 multi-parametric probe (YSI-556 MPS). Measured responses included RNA:DNA ratio, survival, adult and embryo size, and reproduction parameters.
2.2. RNA:DNA ratio estimation

The organisms were collected for biochemical analysis after 7, 14 and 21 days. Each *Daphnia* individual was placed alive in a nuclease-free Eppendorf tube and immediately frozen at −80 °C. Methods for quantification of nucleic acids largely followed the method described in Vrede et al. (2002) with some modifications, as described below. RNA and DNA were extracted and analysed using the fluorochrome RiboGreen in combination with RNase treatment (Gorokhova and Kyle, 2002). A RiboGreen™ RNA Quantitation Kit (Molecular Probes, cat. # R-11490) was used. Further, Ribonuclease A from bovine pancreas (RNase), Triton X-100, Protease from *Bacillus licheniformis* (Type VIII, lyophilised powder, 7–15 units/mg solid) were supplied by Sigma. Nuclease-free water was also supplied by Sigma. DNA standard (SIGMA) sets were prepared from frozen (−20 °C) aliquoted stock. 1 × TE buffer prepared by diluting the 20×TE buffer with nuclease-free water. RiboGreen reagent solution was prepared by diluting the stock solution 200-fold with 1 × TE buffer. The reagent solution was kept dark and cold and was used within a few hours.

RNA and DNA were extracted from individual zooplankters in a solution of 1 × TE buffer containing Triton X-100 (0.1% final concentration) and protease (0.1 mg mL−1 final concentration). Extraction buffer (50 mL) was added to each Eppendorf vial containing one zooplankton individual. The animal was thoroughly mixed with a plastic pellet micropostle (Eppendorf). Another 250 mL of the extraction buffer was added afterwards. Samples were shaken at room temperature on a multiple vial head for 1 h and then spun in a centrifuge for 1 min, plus another 1 min, at 2000 rpm.

Fluorescence measurements were performed using a fluorometer BioTek Synergy HT (filters: 485/20 for excitation and 528/20 for emission) and black solid-flat-bottom microplates (Greiner bioone). In each plate, two replicates with 100 µL of each of the extracted samples, controls and RNA and DNA standards were included. Samples, DNA standards and RNA standards received 100 µL per well of RiboGreen, followed by 5 min incubation in darkness. Then the plate was read using the fluorometer with a sensitivity of 55 (RNA and DNA together, first reading). After the first scan, 25 µL per well of RNase working solution was added and the plate was incubated for 20 min in darkness. After the incubation the plate was scanned again to quantify solely the DNA (second reading). RNase solution was found to increase the background fluorescence, somewhat, and this increase was factored into the calculations (Gorokhova and Kyle, 2002).

2.3. Stage structure

Every four days, the age-specific survival (lx), the proportion of the females surviving to age x (days) and reproduction (mx) rate, and the number of juveniles produced per surviving female between the ages x and x + 1, were computed, from which life-cycle tables were constructed. The net reproductive rate (R0) is the mean number of offspring per individual and it was calculated using the following equation:

\[ R_0 = \sum_{x=1}^{m} l_x m_x \]

The generation length (G; days) was calculated using the following equation:

\[ G = \frac{\sum_{x=1}^{m} l_x x m_x}{R_0} \]

The life-table studies were finished at day 21, after which the intrinsic rates of population increase r (days−1) were calculated (Lotka, 1913). Then r was approximated, according to:

\[ r \sim \frac{\ln(R_0)}{G} \]

2.4. Secondary production

Secondary production was estimated by considering biomass increase in each vessel. Both individual growth (from the beginning to the end of the experimental period) and egg production (from the day 7) were taken into account. As dry weight was not measured, juvenile and adult weights were obtained from the length-weight regressions, as established by Kawabata and Urabe (1998):

\[ \ln W = 3.05 + 2.16 \ln L \]

where W is µg of dry weight, and L is the length in mm.

For length measurements, each individual was photographed under a stereomicroscope (Leica MZ 125, Spain) in order to analyse individual size, using Image-J software (U. S. National Institutes of Health, Bethesda). Total carapace length is defined as the length from the top of the daphnid head to the base of its spine. The biomass of a given stage was obtained by multiplying the abundance of this stage by the weight that was estimated from the respective length-weight regression. The most commonly-used method for measuring secondary production (PR) is the weight increment method that consists of multiplying the weight-specific growth rate of each developmental stage (gi) by its biomass (Bi) (Hirst et al., 2005):

\[ PR = \sum (g_i B_i) + \left( g_i B_f \right) \]

The first term of the equation corresponds to the somatic growth rate of the juvenile population (in the present experimental period from day 1 to day 7), and adult individuals (from the day 7), and the second term corresponds to the specific egg production rate of the females (from day 8 to day 21), and the biomass of the adult females (Bf).

The growth rate (gi) was estimated as follows (modified from: Hirst et al., 2005):

\[ g_i = \ln \left( \frac{W_{exit}}{W_{entry}} \right) \frac{MR}{MR} \]

where \( W_{entry} \) is mean weight of individuals in the day i, \( W_{exit} \) is mean weight of individuals in the day i + 1, and MR is the inverse of the experimental period selected (modified from: Jiménez-Méléro et al., 2013). In this particular case, since the measurements were performed at regular intervals of 7 days, MR is equal to 0.143 days−1.

The specific egg production rates were calculated by using the following equation:

\[ g_f = \frac{W_{eggs}}{W_{females}} \frac{MR}{MR} \]

where \( W_{eggs} \) is calculated by considering the weight of an individual egg multiplied by the average clutch size, and MR is the inverse of the experimental period selected (see above). Egg weight was calculated using the length-weight regressions, as established by Glazier (1992).

When comparing two groups, an unpaired t-test was applied.
Comparisons of three and more groups were performed with a one-way ANOVA or Repeated Measures ANOVA, when suitable. When it was necessary, the data were log (x + 1) transformed to improve normality and variance homogeneities (Zar, 1996). P values < 0.05 were considered significant.

3. Results

The pH was monitored during the experimental period by the AT Control computer (366 measurements). The pH values were different in control and low-pH vessels, throughout the experimental period, being their mean values 8.77 (±0.17) and 7.00 (±0.03), respectively (t = 209.8; p < 0.05). Dissolved oxygen and conductivity were higher in low-pH vessels at day 7, although there were no statistically significant differences between control and low-pH treatment vessels in dissolved oxygen, conductivity and alkalinity at the end of the experimental period (Table 1). At this time, however, differences were found in the amount of food available (phytoplankton cell abundance), that was higher in low-pH treatment vessels than in control vessels.

A factorial ANOVA showed that acidification did not affect to the RNA:DNA ratios (F(1, 79) = 0.022, p = 0.88), which decreased significantly (F(2, 79) = 16.489, p < 0.01) throughout the time (Fig. 1). However, the fall pattern was not the same in both treatments (F(2, 79) = 4.675, p = 0.01): in the acidified treatment this ratio decreases considerably over the time, in the control it seems that stabilizes from the day 14.

There were no significant differences in the survival of D. magna during the experimental period, except at day 21, where the low-pH vessels showed a lower mean survival (t = 2.93, p = 0.03; Fig. 2). Differences in the population parameters R0 and r were found between control and low-pH treatments (vessels) (see Table 2). Although both experimental populations, controls and those growing at pH = 7, have the first reproduction at the same time (9 days), the net reproductive rate was significantly reduced by acidification. Almost double the intrinsic rate of population increase (r) was observed in the control vessels (Table 2), whereas no differences in the generation length (G), were observed between control and low-pH treatments.

In fact, acidification had a significant effect on abundance of neonates from day 13 (ANOVA: F = 3.05, p = 0.02). More than 70% of reduction in the accumulated neonates production occurred in low-pH treatment vessels at the end of the experimental period (Fig. 3).

In relation to adult total length, there were no differences between control (3.49 ± 0.04 mm) and low-pH treatment vessels (3.35 ± 0.24 mm) at the end of the experimental period (t = 2.56; p = 0.12). But, differences were found in embryo size between control and low-pH treatments (0.34 ± 0.04 mm and 0.29 ± 0.05 mm in control and low-pH treatment vessels, respectively; t = 4.697; p = 0.04) at the end of the experimental period.

A repeated measures ANOVA showed a significant effect on secondary production of acidification (F = 24.574; p < 0.01), time of exposure of individuals (F = 103.341; p < 0.01) to that condition and their interaction (F = 22.527; p < 0.01) (Fig. 4). Indeed, secondary production increased gradually throughout the time being significantly higher at the end of the experiment, both in pH = 7 (Bonferroni: p < 0.01) and the control (Bonferroni: p < 0.01).

![Fig. 1. RNA:DNA ratios mean value (±SD) obtained in Daphnia magna individuals from control and low pH vessels during the experimental period.](image)

![Fig. 2. Survival (%) mean value (±SD) obtained in Daphnia magna individuals from control and low pH vessels during the experimental period. * Denotes statistically significant differences between control and low pH vessels (p < 0.05).](image)

### Table 1
Mean values (±SD) of physical-chemical and biological parameters measured during the experimental period. * Denotes statistically significant differences between control and low-pH treatment (p < 0.05).

<table>
<thead>
<tr>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (mS cm⁻¹)</td>
<td>0.84 (0.02)</td>
<td>0.88* (0.01)</td>
</tr>
<tr>
<td>D.O. (mg O₂ l⁻¹)</td>
<td>12.45 (0.94)</td>
<td>14.82* (1.07)</td>
</tr>
<tr>
<td>Alkalinity (meq ml⁻¹)</td>
<td>3.1 (0.7)</td>
<td>4.7 (1.1)</td>
</tr>
<tr>
<td>Food (cells ml⁻¹)</td>
<td>65,600 (4032)</td>
<td>68,287 (2583)</td>
</tr>
</tbody>
</table>

Note: Conductivity and Dissolved Oxygen data on day 14 were missed.
Acidification caused a decline in production, such as can be observed in Fig. 4.

The contribution of every stage analysis (i.e. neonates-juveniles on one hand, and reproductive females on the other hand) on the secondary production was performed using differences on biomass separately throughout the time. In the acidified vessels there was not differences between biomass of neonates-juveniles and females (Fig. 5a). In both stages biomass increased between T1 and T2 and this trend continued in the case of neonates-juveniles but not in the females. Despite of this different trend in T3, no differences were detected between biomass of both stages (Fig. 5a). The same pattern was observed in the control vessels, but in this case the increment of neonates-juveniles’ biomass between T2 and T3 was much higher and significantly different (p < 0.05) to the biomass of females (Fig. 5b). Therefore, although both in control and pH = 7 the biomass of neonates increased over the time, this increment was considerably lower in the acidified vessels (Fig. 5c). In the case of the females no differences were detected between treatments and the increment only was significant between T1 and T2 (Fig. 5d).

### 4. Discussion

The results presented in this study have shown that acidification during 21 days affects negatively *D. magna* population growth rate and neonate production. However, no differences were detected in adult size, nor in RNA:DNA ratio. Previous studies have shown that under limited food conditions, *Daphnia* prioritisation of resource allocation will affect firstly reproduction, then growth and ultimately maintenance and hence survival (Glazier, 1992), but no food limitation occurred during the present experiment. Urabe et al. (2003) reported lower individual growth in *D. magna* exposed to increased CO2 concentration, and found a relationship with a decreased food nutritional quality rather than a food limitation. This issue is especially important when *D. magna* is fed just with one microalgae species (Sterner et al., 1993), in the present experiment the mixed diet was used trying to avoid nutritional deficiencies and an algae culture medium with two phosphorous (P) sources was used raising the P content (Urabe et al., 2003). Nevertheless, no direct nutritional status indexes were measured such as Fatty Acids (FA) composition (Rossoll et al., 2012). So, other indirect effects associated to the algae nutritional status might be interfering in the results. However, it is necessary to point out that adult growth has not seemed to be affected, as does occur with other invertebrates that are exposed to acidification (Carter et al., 2012; Keppel et al., 2012; Stumpf et al., 2011), while a lower embryos size was obtained. Then, an energy deviation strategy could be part of the response. The energy provided by the food probably might have been deviated in order to fight against the physiological consequences of a low-pH environmental condition, instead of being deviated to the reproduction energy compartment, leading to reduced reproduction rates and smaller embryos.

Although the RNA:DNA ratio changed during the growth process, surprisingly, there were no differences between treatments, suggesting that the pH experimental conditions might not induce change in RNA:DNA ratios or even this biomarker is not effective/
Fig. 5. Variation in the biomass (mean) over time. a) Comparison of biomass between stages throughout the time in the acidified vessels; b) Comparison of biomass between stages throughout the time in the control vessels; c) Comparison of neonates biomass between treatments (acidified and control vessels) throughout the time. d) Comparison of adults biomass between treatments (acidified and control vessels) throughout the time. T1 is the period between the beginning of the experiment and the day 7, T2 is the period between the day 7 and the day 14, and T3 is the period between day 14 and day 21. Means predicted by a repeated-measures ANOVA. Bars show confidence levels of 0.95. Significant differences throughout the time are indicated with asterisks in grey. Significant differences at a given time are indicated in black. *p < 0.05; **p < 0.01; ***p < 0.001.
useful to ascertain the effects due to CO2 exposure. In terms of physiology, those results indicate that individuals under control or low-pH conditions have the potential for similar protein synthesis rates. Vrede et al. (2002) have reported that the RNA:DNA ratio in *D. magna* responded within 5 h to food quality and it was highly correlated with the somatic growth rate. It is noteworthy that Gorokhova and Kyle (2002) reported the decrease in RNA:DNA ratio along *Daphnia pulex* Leydig 1869 growth, as in the present work. Those authors stated that growth during juvenile development of *Daphnia pulex* is due primarily to an increase in cell size, while in reproducing females, growth is due to increase in both cell size and number. Similarly, it could be occurring in *D. magna*, confirming a similar pattern for cladocerans. Speekmann et al. (2007), found significant differences between *Acartia tonsa* Dana 1849 RNA:DNA ratios under enough food or starvation conditions; however, they found no correlation between RNA:DNA ratios and egg production. These findings indicate a complex relationship between the energy allocation distribution and this ratio and point out the need to use a multi-biomarker approach, such as the Cellular Energy Allocation CEA, for anticipating the consequences on populations (Mouneyrac et al., 2012).

Fertility was greatly reduced by the low pH conditions. Females should increase the energy requirements of metabolism during oogenesis and brooding and, therefore, the control individuals could be in better physiological state than those grown at pH = 7. That could be reflected in the number of eggs, the egg size and the final number of neonates produced. As the population growth rate integrates life-history responses, it can be used to facilitate the prediction of the impact at higher levels of ecological organisation (Barata et al., 2012). For instance, the assessment of population consequences of toxicant effects on individual life-history traits has been favoured by life-table response experiments (Caswell, 1996; Calow and Forbes, 2003). Significant reduction in r at sub-lethal concentrations, or level of stress, can result in significantly lowered population multiplication rates, even when survival is not affected. That may place the population at risk, either from increased biological pressures or by making it more susceptible to environmental randomness (Raimondi and Mckenney, 2006).

Secondary production calculations indicate that under low-pH values a reduction higher that 50% in secondary production had occurred. The reduction in recruitment and secondary production affected by changeable environmental conditions and/or toxic substances have been reported previously (i.e. Parra et al., 2005; Jiménez-Melero et al., 2013). Hanazato and Dodson (1995) noted that sub-lethal exposures to carbaryl reduced *Daphnia* population growth rate (productivity) by about 15%, which was enough to have significant ecological effects on the lake community. A reduction in *Daphnia* population could lead to a reduction in water quality, and a reduction in the efficiency of energy transfer from phytoplankton to fish, because generally *Daphnia* are replaced by smaller and less-efficient herbivores (Havens and Hanazato, 1993). Even though *D. magna* has been used as an experimental model in the present work and other species such as calanoid copepods are also sensible to acidification in freshwater ecosystems (Havens et al., 1993), similar key-species occur in the oceanic zooplankton community (Aguilera et al., 2013) and might be affected by a potential leak of CCS facilities. For instance, Vehmaa (2012) have reported that under low pH values smaller adult copepods can produce fewer eggs than larger counterparts, which might affect the population structures. That situation, together with the increase of phytoplankton production under a high level of CO2 injection (Schippers et al., 2004), might lead to an exacerbated eutrophication process. The aforementioned ecological consequences of such a likely situation, as generated by a leak, should encourage the decision-makers to think deeply about the Environmental Risk Assessment strategy of those CO2 storage facilities.

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References


Havens, K.E., Hanazato, T., 2013. Zooplankton community responses to chemical
stressors: a comparison of results from acidification and pesticide contamination research. Environ. Pollut. 82, 277–288.


