



## Consequences of a simulated rapid ocean acidification event for benthic ecosystem processes and functions

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### ARTICLE INFO

#### Keywords:

*Amphiura filiformis*  
Carbon Capture and Storage (CCS)  
Ocean acidification  
Ecosystem function  
Bioturbation  
Bioirrigation

### ABSTRACT

Whilst the biological consequences of long-term, gradual changes in acidity associated with the oceanic uptake of atmospheric carbon dioxide (CO<sub>2</sub>) are increasingly studied, the potential effects of rapid acidification associated with a failure of sub-seabed carbon storage infrastructure have received less attention. This study investigates the effects of severe short-term (8 days) exposure to acidified seawater on infaunal mediation of ecosystem processes (bioirrigation and sediment particle redistribution) and functioning (nutrient concentrations). Following acidification, individuals of *Amphiura filiformis* exhibited emergent behaviour typical of a stress response, which resulted in altered bioturbation, but limited changes in nutrient cycling. Under acidified conditions, *A. filiformis* moved to shallower depths within the sediment and the variability in occupancy depth reduced considerably. This study indicated that rapid acidification events may not be lethal to benthic invertebrates, but may result in behavioural changes that could have longer-term implications for species survival, ecosystem structure and functioning.

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### 1. Introduction

The potential and realised impacts of anthropogenic carbon dioxide (CO<sub>2</sub>) emissions on the global environment are now well established (Hoegh-Guldberg and Bruno, 2010), leading to political, social and environmental pressure on governments to reduce carbon emissions. Consequently, many countries have opted to reduce carbon emissions within a limited timeframe (e.g. UK, 60% of 1990 levels by 2050; EU member states, 20% of 1990 levels by 2020; Russia, 15–25% of 1990 levels by 2020; USA, 17% of 2005 levels by 2050; Gough et al., 2010; Stern and Taylor, 2010). A principal method adopted by governments is to partially achieve such reductions through the use of Carbon Capture and Storage (CCS) technologies, a process whereby CO<sub>2</sub> is captured from a point emission source and stored in deep geological formations in order to prevent it from entering the atmosphere. This methodology has been endorsed as a key climate change mitigation option by the Intergovernmental Panel on Climate Change (IPCC, 2005), accelerating the development and implementation of the necessary infrastructure (Gibbins and Chalmers, 2008). Whilst CCS technology has the potential to reduce CO<sub>2</sub> emissions from fossil fuel power

stations by 80–90% (Holloway, 2007), the delivery and storage of large volumes of CO<sub>2</sub> has raised concerns about the potential for stochastic leakage and associated environmental consequences (Blackford et al., 2008). Although probably very small, the risk of leakage remains largely unknown and unquantified (Koorneef et al., 2010), however, it is generally accepted that leakage will occur over time (Hawkins, 2004) and that it could have negative consequences for benthic organisms and communities (Harrison et al., 1995; Thistle et al., 2005, 2007).

The spatial extent of an acidification event will depend on the location of the CCS infrastructure and the nature of the release, making it difficult to form generic opinion and advisory conclusions on the likely impact. Nevertheless, seepage from sub-seabed storage is likely to lead to localised effects (Blackford et al., 2009), and, even where such effects are spatially constrained, there is evidence that significant point-source leaks will also simultaneously affect neighbouring ecosystems (including, for example, aquatic releases affecting terrestrial environments; Baxter et al., 1989). Evidence from naturally occurring CO<sub>2</sub> seeps suggest that associated changes in ocean chemistry (pH, HCO<sub>3</sub><sup>-</sup>, etc.) can lead to pronounced biodiversity shifts, most notably the loss of calcifying organisms (Hall-Spencer et al., 2008; Hendriks et al., 2010). In addition, metabolic activity, fertility, growth and survival have all been shown to be negatively impacted by exposure to acidified seawater across a range of taxa (Fabry et al., 2008; Kroeker et al., 2010; Pörtner and Farrell, 2008). The magnitude and rate of effects vary greatly between species, but all calcifying species studied to

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date have been shown to be negatively affected (Hendriks et al., 2010). Although acidification may not necessarily be lethal, elevated levels of CO<sub>2</sub> will affect many physiological processes and has the potential to lead to trade-offs between maintenance activities, such as respiration, growth or reproduction (Widdicombe and Spicer, 2008). In addition to physiological impacts, exposure to acidified seawater can also influence the activity and behaviour of marine invertebrates (e.g. de la Haye et al., 2011; Simpson et al., 2011), which may have significant consequences for ecosystem functioning.

In marine sediment systems, infaunal macro-invertebrates are particularly important in influencing generative and regenerative microbial-mediated processes, such as nutrient transformation and decomposition, vital to maintaining ecosystem condition (e.g. Emmerson et al., 2001; Godbold et al., 2009; Ieno et al., 2006; Laverock et al., 2011; Marinelli and Williams, 2003; Mermillod-Blondin et al., 2004; Norling et al., 2007). In UK and European shelf sea sediments, the brittlestar *Amphiura filiformis* is highly abundant and, where it is present, can be responsible for up to 80% of particle redistribution below the sediment–water interface (Solan and Kennedy, 2002; Vopel et al., 2003). Reduction in seawater pH has been shown to induce muscle wastage in *A. filiformis* and increase rates of metabolism (Wood et al., 2008), potentially leading to changes in activity levels and burrowing capacity. Given the intimate link between infaunal behaviour and nutrient cycling, any widespread effect on the efficiency of bioturbation activity by *A. filiformis* is likely to have ecological consequences for ecosystem function in shelf sea sediment systems (Solan et al., 2004a, 2012). This study experimentally generated a short-term acidification (to pH 6.5) event to investigate the immediate effects of rapid acidification on benthic processes (bioturbation and bioirrigation) and, in turn, ecosystem functioning (nutrient concentration). Visual observations of burrowing behaviour will also indicate whether there are aspects of behavioural response that, following further investigation, may provide a means to identify the presence and spatial extent of CO<sub>2</sub> leakage in areas dominated by this species.

## 2. Materials and methods

### 2.1. Sediment and fauna collection

Individuals of *Amphiura filiformis* were collected from Plymouth Sound (~15 m water depth, 50°21.05'N, 04°07.8'W and 50°20.7'N, 04°07.78'W) using an anchor grab. Sediment was collected from Cawsand, Plymouth Sound (~15 m water depth, 50°19.8'N, 04°11.5'W) using an anchor grab. Sediment was sieved (500 µm mesh) in a seawater bath to remove macrofauna, allowed to settle to retain the fine fraction and homogenised by stirring, before being added to individual cores (capped PVC cores, 100 mm diameter, 200 mm tall) to a depth of 150 mm and overlain by 50 mm seawater. All cores were held in a recirculating seawater system until they were used in the exposure trials.

### 2.2. Seawater acidification and exposure

CO<sub>2</sub> gas was bubbled through natural seawater (salinity ~35) enabling the gas to dissolve rapidly into solution. Release of CO<sub>2</sub> gas, to maintain the pH, was controlled via a solenoid valve connected to the gas cylinder and monitored using a pH controller (Aqua Digital pH-201, accuracy ±0.1% + 0.02) which was cross checked weekly against values given by a regularly calibrated pH metre (InLab® 413SG, Mettler-Toledo). The reservoir electrodes did not require calibration over the course of the study. Two 1m<sup>3</sup> tanks, one containing the acidified sea water and one containing

ambient seawater were used to acclimatise both the *A. filiformis* and the sediment (including meiofauna and microorganisms) prior to the experiment. Cores containing individuals of *A. filiformis* ( $n = 5$  mesocosm<sup>-1</sup>, density equivalent to 640 individuals m<sup>-2</sup>) or sediment with no macrofauna were positioned randomly in the acclimatisation tanks for 96 h prior to the start of the experiment (Fig. 1). Salinity, temperature and alkalinity in both tanks were monitored three times per week (Monday, Wednesday and Friday) throughout the duration of the experiment. Unmeasured carbonate parameters were calculated from these data using constants supplied by Lueker et al. (2000) and Millero (2010) with CO<sub>2</sub> calc., an application developed by the U.S. Geological Survey Florida Shelf Ecosystems Response to Climate Change Project (Robbins et al., 2010).

### 2.3. Observation of species activity and behaviour

Following the acclimatisation period, sediment and fauna were transferred into rectangular thin-walled (5 mm) Perspex aquaria (33 × 10 × 10 cm, density equivalent to 500 individuals m<sup>-2</sup>). Each aquarium was maintained in a temperature controlled room (10 °C) and supplied with seawater (on a flow through system from the acclimatisation tanks) at the appropriate pH level and at a rate of ~10 ml min<sup>-1</sup> using a peristaltic pump (Watson–Marlow 323). The faunal redistribution of sediment particles was measured non-invasively using a time lapse sediment profile imaging system (f-SPI, following Solan et al., 2004b), optically modified to preferentially visualise fluorescent dyed sediment particles (luminophores, see Maire et al., 2008) housed in a UV illuminated imaging box (32 × 87 × 62 cm with Phillips blacklight, 8 W, Schiffers et al., 2011). The camera (Canon 400D, 3900 × 2600 pixels, i.e. 10 megapixels, effective resolution = 64 × 64 µm per pixel) was set for an

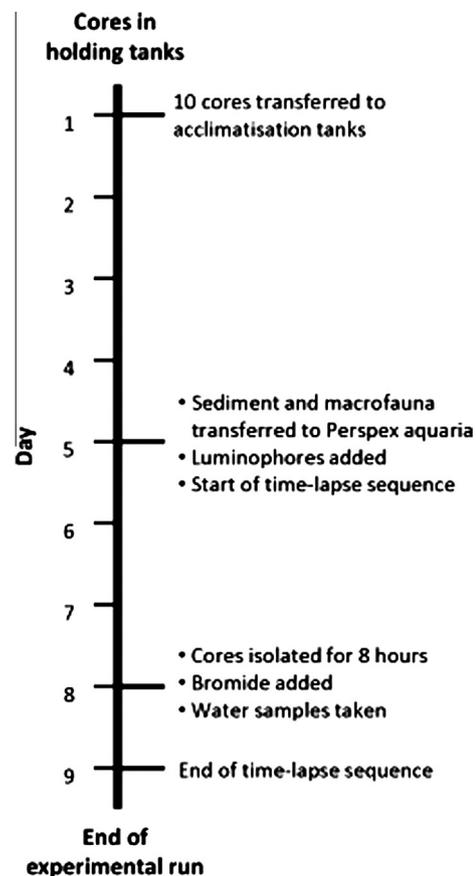


Fig. 1. Timeline diagram of experimental procedure.

exposure of 4s,  $f = 5.6$ , film speed equivalent to ISO 400 and was controlled using third party timelapse software (GB Time lapse, v.2.0.20.0, <http://www.granitebaysoftware.com>). After an acclimatisation period of 24 h to allow macrofaunal establishment within the aquaria, luminophores (Partrac Tracer 2290 pink, size 125–355  $\mu\text{m}$ , 20 g aquaria<sup>-1</sup>) were evenly distributed across the sediment surface immediately prior to the start of each time lapse sequence (1 image 15 min<sup>-1</sup> for 96 h, i.e. 384 images sequence<sup>-1</sup>). Images were saved with colour JPEG (Joint Photographic Experts Group) compression.

Bioirrigation activity was estimated from changes in water column concentrations of an inert tracer, (Sodium bromide, NaBr, dissolved in seawater  $[\text{Br}^-] = 800$  ppm, 5 mM, stirred into the overlying seawater) for 8 h on day 8 of each experimental run, during which time the aquaria were isolated from the seawater supply. Water samples (5 ml) were taken at 0, 1, 2, 4, and 8 h (following Forster et al., 1999; Mermillod-Blondin et al., 2004) and immediately filtered (47 mm  $\varnothing$  GF/F filter) and frozen ( $-18$  °C).  $[\text{Br}^-]$  was analysed using colorimetric analysis using a FIAstar 5000 flow injection analyzer (FOSS Tecator, Höganäs, Sweden). Additional water samples (50 ml, 47 mm  $\varnothing$  GF/F filter) were taken at 0 and 8 h to determine any changes in nutrient concentrations ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_x\text{-N}$ ,  $\text{PO}_4\text{-P}$  and  $\text{SiO}_2\text{-Si}$ ) of the overlying water column and analysed using a nutrient autoanalyser (Branne and Luebbe, AAll).

#### 2.4. Image analysis

The distribution of luminophore particles within the sediment profile was quantified, following Solan et al. (2004b), using a custom made semi-automated macro in ImageJ (v. 1.44), a public domain Java based programme (<http://rsbweb.nih.gov/ij/download.html>). The macro sequentially opens each image and splits it into three separate colour (RGB) channels. The user traces the sediment–water interface (=upper region of interest) using the segmented line tool in the green channel. Identification of luminophores below the sediment–water interface is achieved in the red channel using an appropriate threshold level that distinguishes the luminophore particles from the background sediment. The threshold image is converted to a bitmap (0 = background sediment, 1 = luminophore pixels), allowing the total number of luminophore pixels in each row to be summed for each depth row. In addition, the mean ( $\text{lum}_{\text{mean}}$ ), median ( $\text{lum}_{\text{med}}$ ) and coefficient of variation ( $\text{lum}_{\text{CV}} = \text{standard deviation}/\text{mean}$ ) of the vertical distribution of luminophores recovered from the final image in each sequence were calculated.

#### 2.5. Bioturbation model

A process-based, spatially explicit simulation model (Schiffers et al., 2011) was applied to the timelapse sequence data (1 image 30 min<sup>-1</sup> for 72 h, i.e. 145 images sequence<sup>-1</sup>). Three parameters were estimated; 'activity', the probability that each particle will be displaced at each time step; 'distance', the mean distance a particle is displaced between each time step; and 'tracerdiff', a weighting factor that accounts for any differences in the redistribution probability between tracer (luminophore) and sediment particles. The parameter values are identified by iteratively comparing simulation results to experimental data using summed squares of differences, and a subset of these comparisons across parameter space are compared to check for correlation. The optimal combination is then found by implementing a two-step optimisation process (simulated annealing, followed by Broyden–Fletcher–Goldfarb–Shanno minimisation algorithms (Behzadi et al., 2005; Belisle, 1992; Broyden, 1970; Fletcher, 1970; Goldfarb, 1970; Shanno, 1970) within the 'optim' function in the core

package of the R (v2.13.1) statistical and programming environment (R Development Core Team, 2011).

#### 2.6. Statistical analysis

Following preliminary statistical analysis on the change in bromide concentration across all time points, the change in concentration between 0 and 4 h was analysed, as subsequent time periods showed evidence of tracer equilibration as found elsewhere (e.g. Forster et al., 1999; Mermillod-Blondin et al., 2004). Linear regression models were developed for each of the dependent variables *distance*, maximum luminophore depth ( $\text{lum}_{\text{max}}$ ),  $\text{lum}_{\text{med}}$ ,  $\text{lum}_{\text{mean}}$ ,  $\text{lum}_{\text{CV}}$ ,  $\Delta[\text{Br}^-]$ ,  $[\text{NH}_4\text{-N}]$ ,  $[\text{NO}_x\text{-N}]$ ,  $[\text{PO}_4\text{-P}]$  and  $[\text{SiO}_2\text{-Si}]$ , with levels of pH (6.5 or 8.1) and the presence/absence of *A. filiformis* as independent fixed factors. As a first step a linear regression model was fitted for each dependant variable. Where model validation showed evidence of unequal variance a generalised least squares (GLS; Pinheiro and Bates, 2000; Zuur et al., 2009) mixed modelling approach was used to model the heterogeneity of variance.

All analyses were carried out using the 'nlme' package (v3.1-101; Pinheiro et al., 2011) in the R (v2.13.1) statistical and programming environment (R Development Core Team, 2011).

### 3. Results

Seawater carbonate parameters (Table 1) within the recirculating seawater tanks were stable throughout the duration of the experiment. *A. filiformis* survival was 100% throughout the acclimatisation period and over the course of the experiment. Under acidified conditions individuals displayed emergent behaviour within minutes of exposure (Fig. S1, Time lapse video sequence S1) typical of a stress response to hypoxia (Nilsson, 1999). Oxygen levels in individual aquaria were not measured, however visual examination of the sediment profile did not reveal any evidence (e.g. changes in sediment colour, elevation of redox boundary; Lyle, 1983) of enhanced reduction. This is coherent with previous studies in which oxygen levels were monitored and echinoderms displayed emergent behaviour in response to hypercapnia (e.g. Widdicombe et al., 2009).

#### 3.1. Infaunal-mediated particle redistribution and irrigation

Images from the f-SPI sequences showed active particle reworking in both ambient and acidified treatments, however, behavioural differences observed led to subtle changes in the vertical distribution of luminophores between ambient and acidified conditions (Fig. 2, S2 and 3). A strong correlation was found between the bioturbation parameters *activity* and *distance* (Fig. S3), indicating that there was not a unique global minimum in parameter space. However, as this preliminary optimisation indicated that

**Table 1**

Chemical and physical properties of seawater tanks (mean  $\pm$  1 SD) during the experimental period. pH, salinity, temperature and alkalinity were measured. All other values (pCO<sub>2</sub>; DIC: dissolved inorganic carbon;  $\Omega_{\text{aragonite}}$ : aragonite saturation state;  $\Omega_{\text{calcite}}$ : calcite saturation state) were calculated using CO<sub>2</sub>calc (Robbins et al., 2010) from pH and alkalinity.

	Ambient	Acidified
pH	8.05 $\pm$ 0.07	6.51 $\pm$ 0.06
Temperature (°C)	11.29 $\pm$ 0.46	11.52 $\pm$ 0.55
Salinity (psu)	35.61 $\pm$ 0.23	35.29 $\pm$ 0.34
Alkalinity (mmol l <sup>-1</sup> )	2.63 $\pm$ 0.13	2.43 $\pm$ 0.03
pCO <sub>2</sub> ( $\mu\text{atm}$ )	418.36 $\pm$ 114.24	18325.05 $\pm$ 2421.70
DIC ( $\mu\text{mol kg}^{-1}$ )	2048.92 $\pm$ 72.28	2615.51 $\pm$ 129.11
$\Omega_{\text{aragonite}}$	2.33 $\pm$ 0.26	0.09 $\pm$ 0.01
$\Omega_{\text{calcite}}$	3.64 $\pm$ 0.41	0.13 $\pm$ 0.02

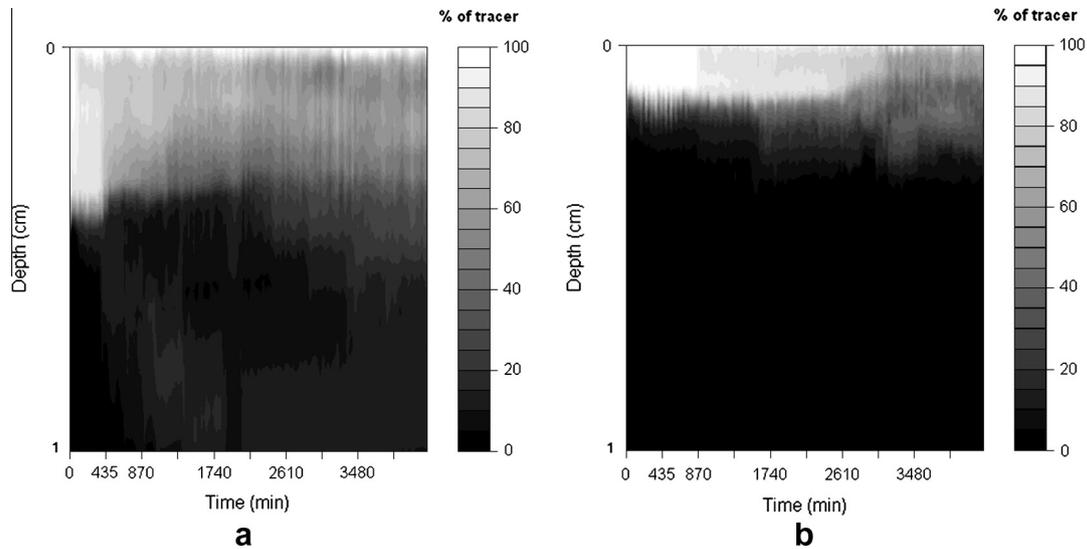


Fig. 2. Representative profiles of luminophore redistribution over 72 h for *Amphiura filiformis* in (a) ambient seawater conditions and (b) seawater acidified with CO<sub>2</sub>.

the optimal value for the parameter *activity* was 0.05, activity was fixed at this value to aid the appropriate optimization of the values for *tracerdif* and *distance*. The mean distance ( $\pm 1$  standard deviation) a particle was displaced (*distance*) was  $0.08 \pm 0.13$  cm for acidified conditions and  $0.03 \pm 0.01$  cm for ambient conditions, although there was no statistical significance between treatments (linear regression: *distance*,  $F = 0.7602$ , d.f. = 6,  $p = 0.41$ , Fig. S4). There was, however, a significant but weak effect of acidification on  $\text{lum}_{\text{max}}$  (linear regression with GLS extension for pH:  $L$ -ratio = 3.8210, d.f. = 1,  $p = 0.05$ ; Model S1, Fig. 3), with deeper mixing occurring in ambient (mean  $\text{lum}_{\text{max}} \pm 1$  standard deviation =  $1.48 \pm 0.25$  cm) relative to acidified (mean  $\text{lum}_{\text{max}} \pm 1$  standard deviation =  $0.41 \pm 1.11$  cm) conditions. No significant difference was detected in either  $\text{lum}_{\text{mean}}$  (linear regression with GLS extension for pH:  $L$ -ratio = 2.0457, d.f. = 1,  $p = 0.15$ ) or  $\text{lum}_{\text{med}}$  after 72 h (linear regression with GLS extension for pH:  $L$ -ratio = 1.1561, d.f. = 1,  $p = 0.28$ ). However, analysis of  $\text{lum}_{\text{CV}}$  revealed much greater variability in ambient relative to acidified conditions (linear regression with GLS extension for pH:  $L$ -ratio = 7.2658, d.f. = 1,  $p < 0.05$ , Model S2, Fig. S4).

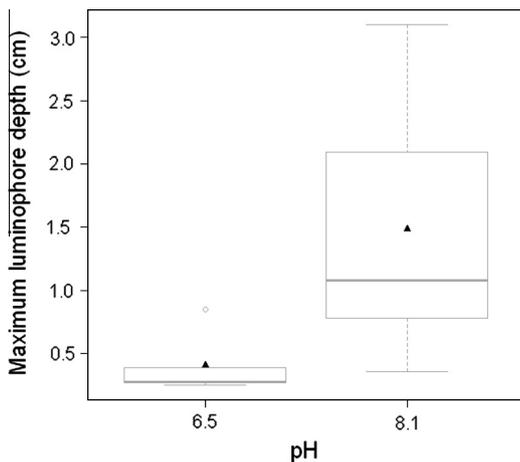


Fig. 3. Effects of seawater acidification on the maximum depth of luminophore redistribution by *Amphiura filiformis*. In each plot the median is indicated by the midpoint, the upper and lower quartiles are indicated by the box, the spread of the data is indicated by the lines and any outliers are represented open circles. Model predictions (linear regression with GLS extension, Model S1) are represented by black triangles.

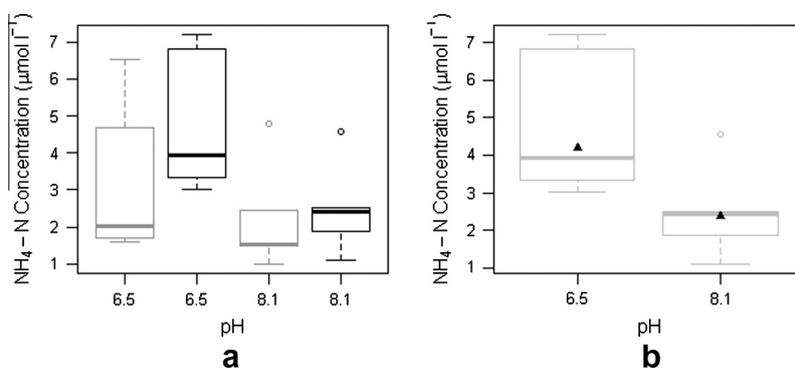
Analysis of  $[\text{Br}^-]$  revealed no significant bioirrigation activity by *A. filiformis* (mean decrease in  $[\text{Br}^-] \pm 1$  standard deviation of  $1.26 \pm 1.94$  mM and  $0.40 \pm 0.70$  mM for *A. filiformis* in acidified versus ambient conditions and  $0.49 \pm 0.67$  mM and  $1.81 \pm 1.52$  mM for aquaria with no macrofauna under acidified versus ambient conditions respectively; Linear regression,  $F = 1.288$ , d.f. = 13,  $p = 0.3125$ , Fig. S5).

### 3.2. Nutrient concentrations

Nutrient concentrations at the start of the experiment did not differ between acidified and non-acidified treatments or between the presence versus absence of *A. filiformis*, indicating that any treatment effects cannot be related to initial conditions (linear regressions,  $[\text{NH}_4\text{-N}]$ ,  $p = 0.6379$ ,  $[\text{NO}_x\text{-N}]$ ,  $p = 0.7561$ ,  $[\text{PO}_4\text{-P}]$ ,  $p = 0.2742$ ,  $[\text{SiO}_2\text{-Si}]$ ,  $p = 0.4327$ ). Analyses carried out on final water column concentrations for each nutrient indicated that the sediment acted as a source for  $[\text{NH}_4\text{-N}]$  (Fig. 4). The concentration of  $[\text{NH}_4\text{-N}]$  was positively affected by acidification (linear regression with GLS extension for pH,  $L$ -ratio = 4.6514, d.f. = 1,  $p < 0.05$ , Model S3, Fig. 4), with increased levels of  $[\text{NH}_4\text{-N}]$  released from the sediment under acidified conditions (mean  $[\text{NH}_4\text{-N}] \pm 1$  standard deviation =  $4.09 \pm 2.15$   $\mu\text{M}$ ,  $n = 10$ ) relative to ambient (mean  $[\text{NH}_4\text{-N}] \pm 1$  standard deviation =  $2.37 \pm 1.33$   $\mu\text{M}$ ,  $n = 10$ ) conditions. However the presence of *A. filiformis* had no discernable additional effect (presence,  $3.68 \pm 2.02$   $\mu\text{M}$ ,  $n = 10$ ; absence,  $2.77 \pm 1.88$   $\mu\text{M}$ ,  $n = 10$ ;  $L$ -ratio = 1.47, d.f. = 1,  $p = 0.22$ ). There was also no significant interaction between *A. filiformis* and the level of acidification ( $L$ -ratio = 0.82, d.f. = 1,  $p = 0.36$ ).

$[\text{NO}_x\text{-N}]$  and  $[\text{PO}_4\text{-P}]$  did not vary greatly within treatments ( $[\text{NO}_x\text{-N}]$ : ambient mean  $\pm 1$  standard deviation =  $3.63 \pm 1.64$   $\mu\text{M}$ ,  $n = 10$ ; acidified mean  $\pm 1$  standard deviation =  $3.46 \pm 0.51$   $\mu\text{M}$ ,  $n = 10$ ;  $[\text{PO}_4\text{-P}]$ : ambient mean  $\pm 1$  standard deviation =  $0.34 \pm 0.09$   $\mu\text{M}$ ,  $n = 10$ ; acidified mean  $\pm 1$  standard deviation =  $0.31 \pm 0.08$   $\mu\text{M}$ ,  $n = 10$ ) and were not affected by the level of acidification or by the presence of *A. filiformis* (linear regressions,  $[\text{NO}_x\text{-N}]$ ,  $F = 0.1159$ , d.f. = 13,  $p = 0.9495$ , Fig. S6;  $[\text{PO}_4\text{-P}]$ ,  $F = 1.055$ , d.f. = 13,  $p = 0.3955$ , Fig. S7).

Both the pH treatment and the presence/absence of *A. filiformis* were found to have an independent effect on  $[\text{SiO}_2\text{-Si}]$  (linear regression with GLS extensions for pH and presence of *A. filiformis*,  $L$ -ratio = 7.5517, d.f. = 2,  $p < 0.05$ , Model S4, Fig. 5).  $[\text{SiO}_2\text{-Si}]$  levels were increased under acidified conditions (mean  $[\text{SiO}_2\text{-Si}] \pm 1$



**Fig. 4.** Effects of seawater acidification on  $\text{NH}_4\text{-N}$  concentrations by *A. filiformis*. In each plot the median is indicated by the midpoint, the upper and lower quartiles are indicated by the box, the spread of the data is indicated by the lines and any outliers are represented open circles. Plot 3a shows all data separated by pH level with aquaria containing *A. filiformis* in black and no macrofauna in grey. Plot 3b represents all data at each pH level with model predictions (linear regression with GLS extension, Model S3) for *A. filiformis* represented by black triangles.

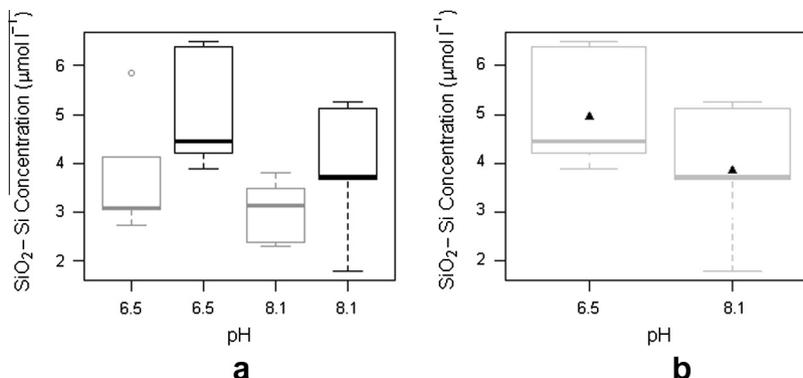
standard deviation =  $4.43 \pm 1.38 \mu\text{M}$ ,  $n = 10$ ) relative to ambient conditions (mean  $[\text{SiO}_2\text{-Si}] \pm 1$  standard deviation =  $3.46 \pm 1.14 \mu\text{M}$ ,  $n = 10$ ) and, in the presence of *A. filiformis*, more  $[\text{SiO}_2\text{-Si}]$  was released into the water column (mean  $[\text{SiO}_2\text{-Si}] \pm 1$  standard deviation =  $4.50 \pm 1.40 \mu\text{M}$ ,  $n = 10$ ) relative to when there were no macrofauna present (mean  $[\text{SiO}_2\text{-Si}] \pm 1$  standard deviation =  $3.39 \pm 1.04 \mu\text{M}$ ,  $n = 10$ ). The presence of *A. filiformis* was the most influential variable ( $L$ -ratio = 4.7150, d.f. = 1,  $p < 0.05$ ), followed by seawater acidification ( $L$ -ratio = 3.5575, d.f. = 1,  $p = 0.0593$ ), although both of these effects were weak. No interaction was detected between the variables.

#### 4. Discussion

This study demonstrated that *A. filiformis* is capable of surviving short-term exposure to acidification, although individuals did exhibit emergent behaviour analogous to stress responses observed elsewhere (e.g. hypoxia, Nilsson, 1999). This is consistent with other studies which have indicated that a number of marine species are capable of surviving acute exposures to acidification (Donohue et al., 2012; Pörtner et al., 2004; Small et al., 2010; Widdicombe and Needham, 2007). However, previous work has demonstrated that a variety of changes in the abiotic environment affect species behaviour and, subsequently, nutrient turnover and primary production in marine sediment systems (Biles et al., 2003; Dyson et al., 2007; Godbold et al., 2011; Bulling et al., 2008, 2010; Langenheder et al., 2010; Hicks et al., 2011). It is also known that context-dependent changes to organism physiology

pre-empt measurable changes in a species functional capacity within an ecosystem (Widdicombe and Spicer, 2008; Hughes et al., 2010; Fehsenfeld et al., 2011); indeed, echinoderms lack an ability to fully compensate for acidification through increasing the bicarbonate level of extracellular fluid (Miles et al., 2007; Spicer et al., 2011) and exposure to acidified seawater has been shown to affect the protein, lipid and carbohydrate levels in the pyloric caeca of the sea star *Luidia clathrata* (Schram et al., 2011). Reduced protein synthesis (Langenbuch et al., 2006), inferred muscle wastage (Wood et al., 2008), reduced growth rates (Berge et al., 2006) and immunosuppression (Hernroth et al., 2011) have all been documented as responses to seawater acidification for marine invertebrates. Hypercapnia is known to suppress metabolism in several species (e.g. Widdicombe and Spicer, 2008) and causes lethargy in the ophiuroid *Ophioereis schayeri* at pH 7.8 (Christensen et al., 2011), which may lead to reductions in activity levels and impair the performance of routine behaviour. At lower pH levels (pH 7.6–7.4), however, compensatory mechanisms appear to be activated in *O. schayeri* as oxygen uptake increases coinciding with copious secretion of mucous, a known stress response. Oxygen consumption is also up-regulated under acidified conditions in *A. filiformis* (Wood et al., 2008) and in the arctic ophiuroid *Ophiocten sericeum* (Wood et al., 2011), suggesting that individuals attempt to maintain normal levels of activity.

Whilst the observed onset of emergent behaviour most likely reflects a response to hypercapnic conditions rather than other known causes of stress, such as hypoxia (Rosenberg et al., 1991), behavioural changes in response to the onset of acidification do



**Fig. 5.** Effects of seawater acidification on  $\text{SiO}_2\text{-Si}$  concentrations by *A. filiformis*. In each plot the median is indicated by the midpoint, the upper and lower quartiles are indicated by the box, the spread of the data is indicated by the lines and any outliers are represented open circles. Plot 4a shows all data separated by pH level with aquaria containing *A. filiformis* in black and no macrofauna in grey. Plot 4b represents all data at each pH level with model predictions (linear regression with GLS extension, Model S4) for *A. filiformis* represented by black triangles.

occur rapidly and evidence is emerging that altered behaviour may modify organism–sediment and community interactions (Briffa et al., 2012). In the present study, it is clear that individuals of *A. filiformis* moved to shallower depths within the sediment profile under acidified conditions and that the variability in the depth of occupancy reduced relative to ambient conditions, yet these changes in behaviour were insufficient to cause demonstrable effects on functioning. Whilst it is possible that we may not have detected a strong affect because the response behaviour of *A. filiformis* forms an extension of normal behaviour (Solan and Kennedy, 2002; O'Reilly et al., 2006), we interpret our findings to be a reflection of the short duration of our experiment. If this is the case, the observed changes to species behaviour could be extremely important over longer timescales because they are likely to lead to secondary effects, such as increased (Bibby et al., 2007) or decreased (Dixon et al., 2010) predator evasion, reduced responses to olfactory cues (Cripps et al., 2011) and decreased locomotion (de la Haye et al., 2011), all of which affect post-acidification survival and/or the contribution that individual species make to ecosystem functioning over the longer term (Bulling et al., 2010). Where sub-lethal predation of *A. filiformis* arms by demersal fish species is extensive (Bowmer and Keegan, 1983), for example, the emergent behaviour reported here will render the population more vulnerable to predation, strengthening the trophic interconnection between benthic and pelagic food webs. Furthermore, changes in sediment turnover, resulting from decreased or altered bioturbation activity, will affect microbial activity and, in turn, has the potential to affect major pathways of biogeochemical cycling (Gilbertson et al., 2012).

It is important to consider changes in bioirrigation activity, as well as changes in behaviour that affect particle redistribution. The observed increases in ammonia and silicate concentrations cannot be attributed to increased bioirrigation activity, but it is likely that observed changes in nutrient concentrations, albeit small, indicate the start of changes in microbial activity and composition, particularly in terms of the realised ratio of archaea to bacteria (Wyatt et al., 2010; Gilbertson et al., 2012). Indeed, microbial nitrification rates have been demonstrated to decrease under experimentally reduced pH conditions (Beman et al., 2010). In particular ammonia oxidation rates are strongly inversely correlated with pH and have been found to be reduced by up to 90% at pH 6.5 and completely inhibited at pH 6 (Huesemann et al., 2002; Kitidis et al., 2011) in the water column, although rates of ammonia oxidation within the sediment profile are not necessarily affected (Kitidis et al., 2011, Laverock et al., unpub.). It should be noted, however, that not all changes in biogeochemical cycles are attributable to the direct effects of acidification on the microbial community. In the case of silicate, for example, acidification of seawater may accelerate the chemical breakdown of diatom tests, leading to an increased rate of silicate release. The bioturbation activity of burrowing macrofauna has been previously shown to have a significant effect on sediment silicate fluxes (Olgard et al., 2008) through increased mixing across the sediment water interface.

Within the context of acidification events associated with CO<sub>2</sub> leakage from a subsea carbon storage site, even short-term localised events have the potential to lead to secondary effects that have functional consequences at larger scales and over longer timescales. Here, we have shown that a functionally important bioturbator (Solan and Kennedy, 2002; Wood et al., 2009) switches behaviour in response to acidification. Changes in species behaviour could also lead to shifts in the benthic community composition. Polychaetes, for example, have been shown to be less sensitive to seawater acidification (Widdicombe and Needham, 2007), and may become more competitive under hypercapnic conditions. It is also possible that species, such as *A. filiformis*, that exhibit emergent behaviour, may become more susceptible to

predation or displacement, especially if an acidification event coincides with high current flow (Loo et al., 1996; Solan and Kennedy, 2002) or times of high predator abundance (Pape-Lindstrom et al., 1997), affecting energy flow through the food web (O'Connor et al., 1986; Lawrence, 2010).

Whilst there are demonstrable physiological and ecological consequences of an acidification event, the likelihood of occurrence and regional community responses of benthic assemblages have not been estimated. Identifying and then monitoring any release of CO<sub>2</sub> from sub-seabed carbon storage sites will be critical in assessing their success as a long-term option for reducing CO<sub>2</sub> atmospheric emissions (Lenzen, 2011). CCS sites are obliged to maintain a leakage rate of 0.01% or less per year to ensure that any associated rise in global temperature is negligible (Lenzen, 2011), yet even at these low levels the local impact of gas release could be considerable. Accurately measuring subtle changes in carbonate chemistry remains difficult in the field and is not yet tractable to monitor remotely. Notwithstanding the need for appropriate monitoring tools (e.g. biomarkers, Hardege et al., 2011), there is scope to monitor behavioural responses of species that show particular behaviours in response to acidification. This approach could prove to be a cost effective method to monitor large areas of seabed, although understanding how benthic species respond to such events is still in its infancy and will need continued investment.

#### Acknowledgements

The authors would like to thank the crew of the MBA Sepia for assistance in animal, sediment and seawater collection, Amanda Beesley and Malcolm Woodward for analysing water samples and the technical support staff at PML. This study was funded by NERC studentship (NE/H524481/1) awarded to FM. This paper is also a contribution to "Sub-seabed carbon storage and the marine environment" (ECO<sub>2</sub>) a Collaborative Project funded under the European Commission's Framework Seven Programme Topic OCEAN.2010.3.

#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.marpolbul.2012.11.023>.

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