

# Effects of CO<sub>2</sub> induced seawater acidification on infaunal diversity and sediment nutrient fluxes

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**ABSTRACT:** A mesocosm experiment was conducted to quantify the effects of short- (2 wk) and long-term (20 wk) exposure to acidified seawater on the structure and diversity of macrofaunal and nematode assemblages in 2 different sediment types. The impact of acidified seawater on sediment nutrient fluxes was also determined. Using carbon dioxide (CO<sub>2</sub>) gas, seawater was acidified to pH 7.3 (mimicking ocean acidification), 6.5 or 5.6 (mimicking leakage from a sub-seabed CO<sub>2</sub> store site). Control treatments were maintained in natural seawater (pH ≈ 8.0). Exposure to acidified seawater significantly altered community structure and reduced diversity for both macrofaunal and nematode assemblages. However, the impact on nematodes was less severe than that on macrofauna. While the communities in both sediment types were significantly affected by changes in seawater pH, impacts on sandy sediment fauna were greater than those on muddy sediment fauna. Sandy sediments also showed the greatest effects with respect to nutrient fluxes. In sand, the efflux of nitrite, nitrate and silicate decreased in response to increased acidification while the efflux of ammonium increased. In mud, acidification increased the efflux of ammonium but had no effect on the other nutrients. We conclude that both leakage from carbon storage and ocean acidification could cause significant changes in the structure and diversity of coastal sediment communities. Lowered seawater pH could also affect nutrient cycling directly by altering bacterial communities and indirectly through impacts on the abundance and activity of key bioturbators.

**KEY WORDS:** Biodiversity · Macrofauna · Meiofauna · Ocean acidification · Nutrient flux · Carbon storage

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## INTRODUCTION

Atmospheric emissions of carbon dioxide (CO<sub>2</sub>) diffuse passively into global ocean surface waters and result in an increase in partial pressure of the gas coupled with reduced pH, first in surface waters and subsequently at greater depths. Compared to pre-industrial times, seawater pH has fallen by 0.1 unit, indicating a 30% increase in the concentration of H<sup>+</sup> ions (Caldeira & Wickett 2003), while the current rate of acidification is 0.015 pH units per decade (Haugan & Drange 1996). As further emissions of CO<sub>2</sub> are inevitable, it is reasonable to assume that concentrations of atmospheric CO<sub>2</sub> will continue to rise and pre-

dictions are that they will exceed 1500 ppm sometime between 2100–2200 (Pörtner et al. 2004). More immediately, the Intergovernmental Panel on Climate Change (IPCC) has predicted that levels could reach 800 ppm by 2100 (Feely et al. 2004). As a result of these increases, it is possible that the average pH of surface water could fall by up to 0.4 units before 2100 and a reduction of 0.7 units could occur by 2250 (Caldeira & Wickett, 2003). However, effects of ocean acidification on seawater chemistry can already be seen in some coastal ecosystems. Feely et al. (2008) observed corrosive seawater (i.e. undersaturated with respect to aragonite) upwelling onto large portions of the North American Pacific continental shelf. Although seasonal

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upwelling is a natural phenomenon, ocean acidification has increased the size of the area exposed to corrosive waters.

Political, social and environmental pressures to reduce CO<sub>2</sub> emissions have led several governments to seek new options for CO<sub>2</sub> mitigation. One such option involves injecting CO<sub>2</sub> into underground porous reservoir rocks (Holloway 2005), which is known as geological storage. This technique has been in use at the Sleipner West gas field in the Norwegian sector of the North Sea since 2000 where around  $1 \times 10^6$  tons of CO<sub>2</sub> are currently being sequestered each year (Holloway 2005). At the Gleneagles Summit in July 2005, the leaders of the world's major economic powers (Group of Eight: Canada, Italy, France, Germany, Japan, Russia, UK, USA) declared they would 'work to accelerate the development and commercialization of CO<sub>2</sub> capture and storage (CCS) technologies'. At ~USD140 million, the total budget for CCS research and development in Europe and North America in 2005 was substantial (Tjernshaugen 2007) and geological storage is considered a practical tool in reducing emissions (Gibbins et al. 2006). While it is assumed that storage sites would be selected to minimize the potential for leakage, subsurface storage leaks are possible over time (Hawkins 2004) and may have a considerable effect on marine ecosystems (Blackford et al. in press). Despite this, little is known about long-term issues which may arise from underground storage of CO<sub>2</sub>. Data are therefore urgently needed to quantify the potential impact of leakage on marine organisms and ecosystem processes (Raven et al. 2005).

Sediments are characterised by strong geochemical gradients and the pH of pore waters at depths of 30 cm may be as much as 1 pH unit lower than in the overlying water (Fenchel & Riedl 1970). With such geochemical variability, it is difficult to imagine how benthic sediment systems could be affected by the relatively small pH changes resulting from increased atmospheric CO<sub>2</sub> and subsequent ocean acidification. However, benthic communities are strongly stratified, with different species and size groups characteristically occupying different sediment strata (Barnes & Hughes 1988). The oxygen-rich surface layer is the most densely inhabited sediment region and is home to the majority of multi-cellular, infaunal organisms. Only those species capable of oxygenating their immediate environment are able to dwell below the redox discontinuity depth (Kristensen 2001). The presence of these depth-constrained niches means that although benthic systems as a whole are already subject to a relatively large range in pH, many of the organisms and processes that exist within them are not.

While the majority of macrofaunal species are restricted to the upper oxic layers, others are able to

inhabit sediment below this layer. They do so by constructing burrows which they irrigate with oxygenated water from above. These burrows, and their occupants, experience significant fluctuations in pH (as much as 2 pH units) and dissolved oxygen concentration (between saturation and near anoxia) generated by the periodic ventilation of burrows (Kristensen 2001). Such oscillations are absent or weak at the water–sediment interface. Consequently, burrowers may have greater tolerance to changes in pH than non-burrowers living near the sediment surface. In addition, recent work has already identified significant variability in pH sensitivity in a number of different benthic taxa (e.g. Miles et al. 2006, Spicer et al. 2006, Pane & Barry 2007, Widdicombe & Needham 2007). Even among organisms which depend on calcium carbonate structures, variability in tolerance has been observed, with echinoderms showing less tolerance to pH change than molluscs (Shirayama & Thornton 2005) and crustaceans (Spicer et al. 2006). This potential difference in pH tolerance among different benthic species could lead to the selection of more tolerant species, thereby changing the structure and function of sediment communities in the face of changing levels of pH.

In addition to potential changes in the diversity and structure of benthic communities, changes in seawater pH could also have a significant impact on one of the key functions performed by coastal sediment ecosystems: nutrient cycling. Despite the potentially large ecosystem effects that would result from changes in the cycling of nitrogen, phosphorous and silicon, very little quantitative data exists to determine the precise impacts of seawater acidification on the transport of these nutrients. To date, only one experimental study has addressed this subject (Widdicombe & Needham 2007) and evidence from this paper suggests that increased seawater acidification may have significant implications for sediment nutrient flux. However, sediment flux varies considerably among sediments with different microbial communities, organic content, granulometry and faunal communities. Additional studies are therefore needed to identify the likely response of nutrient cycling in different sediments.

This study uses a mesocosm-based experiment to demonstrate and quantify the impacts of CO<sub>2</sub> induced seawater acidification on the diversity and structure of 2 different sediment communities. It also determines impacts on sediment flux for key nutrient species.

## MATERIAL AND METHODS

**Collection of sediment and associated fauna.** From 9 to 12 May 2005, sediment was collected from 2 sites, one muddy and one sandy, situated in the middle of

Table 1. Description of sediment collection sites. Sediment data are mean  $\pm$  SD

	Site 1	Site 2
Name	Gray Island Sound	Solbergstrand
Geographical position	59°41.988'N, 10°31.506'E	59°36.768'N, 10°38.709'E
Sediment type (Wentworth)	Silt	Very fine sand
Median grain size ( $\mu\text{m}$ )	14.60 $\pm$ 0.75	80.75 $\pm$ 3.73
% silt/clay	85.5 $\pm$ 1.5	36.0 $\pm$ 3.3
% total carbon	2.86 $\pm$ 0.08	0.684 $\pm$ 0.10
% organic carbon	2.76 $\pm$ 0.11	0.511 $\pm$ 0.04
% total nitrogen	0.231 $\pm$ 0.01	0.045 $\pm$ 0.01
% organic nitrogen	0.225 $\pm$ 0.01	0.031 $\pm$ 0.01
Water depth (m)	36	25

the Oslofjord, Norway (Table 1). Samples were collected at each site and analysed for sediment granulometry, carbon and nitrogen content.

At Site 1 (the muddy site), 45 undisturbed cores were collected by sub-sampling from a 0.1 m<sup>2</sup> box corer. In each box-core sample, a stainless steel circular core (26 cm  $\varnothing$ ) was pushed into the sediment to a depth of 40 cm. Each circular core was then removed intact from the box core and transferred to a pre-seasoned food grade plastic bucket (30 cm  $\varnothing$ , 40 cm deep).

At Site 2 (the sandy site), 45 buckets (30 cm  $\varnothing$ , 40 cm deep) were filled to ~40 cm depth with sediment collected using a 0.1 m<sup>2</sup> vanVeen grab. It was not possible to collect undisturbed cores at this site as the box corer could only penetrate to a depth of 5 to 10 cm. Although the collection of undisturbed cores is ideal, the use of grab-collected sediment in experiments on soft sediment communities has been shown to be a credible method of sediment collection (Widdicombe & Austen 1998, 1999, 2001, Widdicombe et al. 2004).

Cores were transferred to the benthic mesocosm of the Norwegian Institute for Water Research (NIVA)

facility at Solbergstrand, Oslofjord. While being transferred to the mesocosm, the cores from both sites were covered with seawater to prevent desiccation and minimise temperature change. Once in the mesocosm, the buckets were placed in a flow-through holding basin filled with seawater to a depth of 1 m. A pipeline situated at 60 m in the adjacent fjord continuously supplied the holding basin with natural seawater.

**Experimental set up and sampling protocol.** After several days in the holding basin, 40 buckets from each of the 2 sites were transferred to the experimental system (Fig. 1) and each bucket was supplied independently with natural seawater, via a delivery tube, at a rate of ~70 ml min<sup>-1</sup>. Each of the 4 pH treatments (see Table 2) contained 10 buckets from each of the 2 sites. Allocation of buckets to a particular pH treatment and the positioning of each bucket within the pH treatment systems were random. The 10 unallocated buckets (5 from each site) remained in the holding basin where they were sampled on 15 May to determine their sediment carbon and nitrogen content.

Seawater acidification in the experimental system began on 16 May 2005. To reduce the impact of an acute acidity shock, the pH was gradually lowered to the required treatment values over a period of 1 wk. The nominal pH treatment levels were reached on 23 May and this date was taken as the start of the experimental exposure period. Seawater acidification followed the methods described in Widdicombe & Needham (2007).

Two weeks after the completion of seawater acidification (6 June 2005), 10 buckets (5 from each site) from each pH treatment were randomly selected. The water supply to these buckets was terminated and the time noted. This was deemed to be the start of the nutrient incubation period ( $t_0$ ). At  $t_0$  a 50 ml water sample was drawn from the water overlying each core, GF/F filtered (47 mm  $\varnothing$ ) and stored in an acid-washed Nalgene bottle. Samples were stored frozen until analysis with an autoanalyser (AAIII) Bran & Luebbe, for

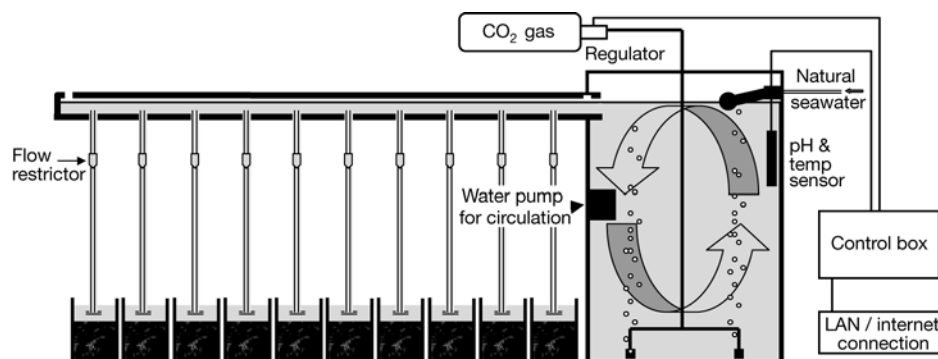


Fig. 1. Experimental set-up showing side view of a seawater acidification system including header tank and exposure buckets

ammonium, nitrate, nitrite, silicate and phosphate concentrations using standard methods (Brewer & Riley 1965, Grasshoff 1976, Mantoura & Woodward 1983, Kirkwood 1989, Jia-Zhong & Jie 2002). Further samples were taken from each bucket after 6, 12, 18 and 24 h. Stirring of the overlying water was achieved by gently bubbling air through a tube (2 cm  $\varnothing$ ) suspended 1 cm above the sediment surface in the centre of the bucket. After the final water sample was taken, a small core of sediment (1.6 cm  $\varnothing$ , 6 cm deep) was collected in each bucket and dried at 80°C for 12 h. The dried sediment was ground and stored in moisture-proof plastic vials before being analysed for carbon and nitrogen content. Aliquots of the powdered sediment were weighed into aluminium capsules for total carbon and silver capsules for organic carbon. Inorganic carbonates were removed from the samples in silver capsules by addition of 2 drops of sulphuric acid (Verardo et al. 1990). The capsules were dried at 60°C for 48 h, crimped and analysed on a Thermo Finnegan Flash EA1112 elemental analyser using acetanilide as a calibration standard.

Simultaneous with carbon and nitrogen sampling, 4 small (1.6 cm  $\varnothing$ , 6 cm deep) randomly placed cores were taken from each bucket and pooled in a 250 ml container. These samples were preserved with 10% formalin and brought to the Plymouth Marine Laboratory (PML) for meiofauna extraction. Meiofauna were extracted by flotation using a suspension of colloidal silica (Ludox) with a specific gravity of 1.15. Meiofauna were collected using a 63  $\mu$ m sieve and extraction was repeated 5 $\times$ . Extracted nematodes were suspended in a mixture of 5% glycerol, 20% ethanol and water, which slowly evaporated overnight on a warming plate to pure glycerol, and then mounted on microscope slides. Nematodes were identified to the lowest possible taxonomic level using pictorial keys (Warwick et al. 1998). The remaining sediment in each bucket was sieved over a 0.5 mm mesh and the residue was transferred to a 500 ml container and preserved with 10% formalin. On returning to PML, the macrofauna were extracted from this residue under a stereo microscope and identified to species or the lowest taxonomic level possible.

The remaining buckets were exposed to their assigned pH treatments for a further 18 wk and subsequently sampled for nutrient flux rates, carbon and nitrogen content, macrofaunal and meiofaunal community structure and diversity during 12 to 14 October 2005, for a total exposure of 20 wk. At this time, a sediment pH profile was constructed for each core. Using a WTW

pH340i meter with a Hamilton electrode, measurements were taken in the water above the sediment at the sediment surface and at 1 cm intervals within the sediment down to a depth of 6 cm (Fig. 2). These profiles demonstrated that acidification was not restricted to the overlying water and the top surface layers. This transmission of pH effect down through the sediment was also observed by Dashfield et al. (2008) who used the same method to assess the impact of seawater acidification on sediment infauna.

**Monitoring of experimental conditions.** Both temperature and pH of the reservoir tank water (Fig. 1) were constantly monitored throughout the experimental period using the Walchem Webmaster-GI controller via 4 pH electrodes (S650CD), one being placed in each reservoir. In addition, point measurements of temperature, pH, alkalinity and oxygen saturation levels in each of the 4 reservoir tanks were taken every 2 to 3 d (Table 2, reservoir). At the same time point measurements of temperature and pH were also taken from 20 randomly selected buckets (5 from each of the 4 pH treatment levels) (Table 2, bucket). Temperature and pH were measured using a Jenway 3150 pH meter, alkalinity using a single point titration and oxygen saturation using a WTW Oxi 340i oximeter.

**Statistical analysis.** To determine whether seawater acidification caused changes in macrofaunal and meiofaunal community structure, multivariate analyses were performed using PRIMER 6 (Clarke & Gorley 2006). Similarity values (Bray-Curtis) were calculated between pairs of samples and analyses were conducted on both untransformed and presence-absence data as the 2 transformations identify responses in different aspects of the community (Clarke & Green 1988). Analyses using untransformed data identify

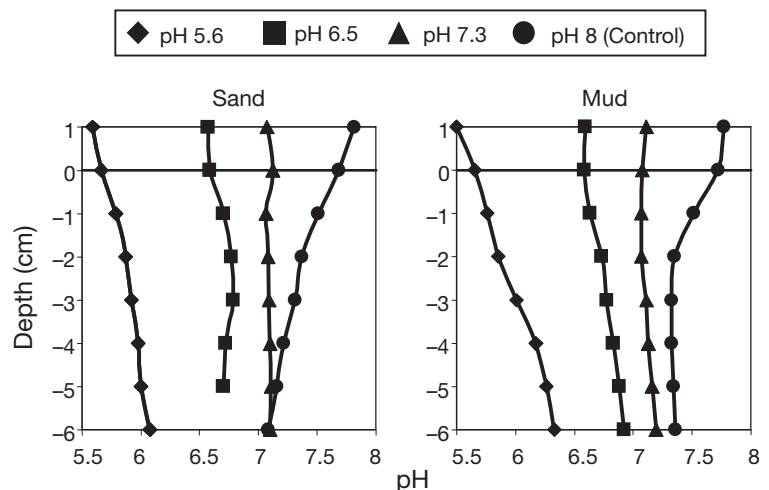


Fig. 2. Sediment pH profiles with depth in sand and mud under different levels of seawater acidification

Table 2. Chemical and physical properties of reservoir and bucket water (mean  $\pm$  1 SD) during the experimental exposure period

Treatment (pH level)	pH	Temp (°C)	O <sub>2</sub> (% saturation)	Alkalinity (mmol l <sup>-1</sup> )
<b>Reservoir</b>				
Control (8.0)	8.01 $\pm$ 0.07	7.8 $\pm$ 0.7	81.65 $\pm$ 3.32	2.48 $\pm$ 0.11
7.3	7.23 $\pm$ 0.11	8.4 $\pm$ 0.6	82.75 $\pm$ 3.48	2.52 $\pm$ 0.05
6.5	6.56 $\pm$ 0.11	8.4 $\pm$ 0.6	80.36 $\pm$ 3.57	2.49 $\pm$ 0.10
5.6	5.70 $\pm$ 0.26	8.4 $\pm$ 0.6	78.00 $\pm$ 3.73	2.51 $\pm$ 0.06
<b>Bucket</b>				
Control (8.0)	7.99 $\pm$ 0.04	8.5 $\pm$ 0.6		
7.3	7.22 $\pm$ 0.10	9.3 $\pm$ 0.7		
6.5	6.58 $\pm$ 0.10	9.0 $\pm$ 0.6		
5.6	5.64 $\pm$ 0.05	9.0 $\pm$ 0.6		

changes in the relative abundances of the numerically dominant species, while the use of presence–absence data indicates species addition and/or loss which can indicate changes in species diversity (richness component).

The analysis used a factorial design, with 3 crossed, fixed factors: sediment source (2 sites), pH level (4 levels) and exposure period (2 durations). Formal testing of 2- and 3-way interactions among these factors, distinguished from their main effects, cannot be carried out by the non-parametric routines in PRIMER (interaction being a parametric, linear model-based concept), but can be performed with the permutational MANOVA procedures introduced by McArdle & Anderson (2001) and Anderson (2001). Inevitably, permutational multivariate ANOVA (PERMANOVA) requires somewhat more restrictive assumptions than a fully non-parametric approach (viz. linear model, additive error, constant variance), but crucially operates on the similarity or dissimilarity matrix of choice (e.g. Bray-Curtis), and avoids unrealistic normality (or other distributional) assumptions. It does this by exploiting permutation to generate null hypothesis distributions for its pseudo- $F$  statistic, the latter being constructed by exact analogy with the standard  $F$  statistic for corresponding univariate ANOVA designs. Here, we used the PERMANOVA+ routines (beta version; Anderson et al. 2008), which are an 'add-in' to the PRIMER 6 software, to carry out formal tests for main effects and interactions among the 3 factors. The presence of interactions necessitates the more piecemeal treatment of the different sediment sources, exposure periods and pH levels, so overall effects of pH and time (exposure period) on community structure were tested for using the non-parametric 2-way analysis of similarities (ANOSIM) (Clarke 1993). Where significant overall effects existed, 1-way pairwise ANOSIM was used to identify pH treatment-specific effects.

For each sample, the number of species ( $S$ ), number of individuals ( $NI$ ) and Pielou's evenness ( $J'$ ) were calculated for both macrofauna and meiofauna and plotted against seawater pH. Visual inspection of these plots indicated that it was necessary to transform the data for both  $S$  and  $NI$  prior to fitting ANOVA and regression models to ensure approximate homogeneity of variance. The transformations chosen were  $\sqrt{S}$  and  $\log_{10}NI$ , in keeping with past experience: species richness often exhibits a Poisson distribution, for which a square root transformation will break the automatic link between the variance and the mean, while the distribution of total abundance is usually log normal, a log transformation thus restoring

normality (Clarke & Warwick 2001). To test for the presence and nature of any relationship of  $\sqrt{S}$ ,  $\log_{10}NI$  or  $J'$  with seawater pH, decreasingly constrained models ( $H_0$ ) were compared against a fully unconstrained model ( $H_1$ ). These constrained models were:

$H_0$ :  $y = \mu$  (assumes no relationship, i.e. a single mean, and fits 1 parameter)

$H_0$ :  $y = \alpha_1 + \beta x$  (assumes a linear relationship and fits 2 parameters)

$H_0$ :  $y = \alpha_1 + \beta x + \gamma x^2$  (assumes a quadratic relationship and fits 3 parameters)

The fully unconstrained model ( $H_1$ ) assumed that each treatment level had a separate mean. This was essentially the ANOVA model and fitted 4 parameters. The  $F$ -ratio was calculated as:

$$F = \frac{(RSS_{H_0} - RSS_{H_1}) / (p - q)}{RSS_{H_1} / (n - p)} \quad (1)$$

where  $RSS$  is the residual sum of squares from fitting the model,  $p$  is the number of parameters fitted under  $H_1$ ,  $q$  is the number of parameters fitted under  $H_0$ , and  $n$  is the number of sample points. If the calculated  $F$ -value is less than  $F$  at the 5% level,  $H_0$  is rejected. A rejection meant that the fit of the constrained model to the data was significantly worse than that of the fully unconstrained model.

The effects on  $\sqrt{S}$ ,  $\log_{10}NI$  and  $J'$  by the communities being placed and maintained in the mesocosm were assessed by comparing initial, 2 wk controls and 20 wk controls using 1-way ANOVA with Fisher pairwise comparisons.

Nutrient flux rates were determined by plotting nutrient concentrations ( $\mu\text{mol}$ ) against incubation time (min) to determine the slope of the relationship. Measurements of nutrient concentration were corrected to allow for the removal of water during sampling following the methods of Townsend (2006). Final fluxes were calculated using:

$$\text{Flux } (\mu\text{mol m}^{-2} \text{ d}^{-1}) = \frac{\text{Slope}}{\text{Area}} \times 10000 \times 60 \times 24 \quad (2)$$

where area is the sediment surface area ( $\text{cm}^2$ ).

In the same way that  $\sqrt{S}$ ,  $\log_{10}NI$  and  $J'$  were tested, the presence and nature of any relationship between seawater pH and nutrient fluxes were determined by again comparing decreasingly constrained models ( $H_0$ ) against a fully unconstrained model ( $H_1$ ).

Two-way ANOVA tests were used to identify any significant effects of either time or seawater pH on the percentage of total carbon, inorganic carbon, organic carbon, total nitrogen and organic nitrogen in both the muddy and sandy sediments.

## RESULTS

### Impact of seawater acidification on macrofaunal community structure and diversity

A PERMANOVA, performed on untransformed and presence/absence macrofaunal community data and based on Bray-Curtis similarities (Table 3), shows that all main effects, 2-way and 3-way interactions, were significant (at least at  $p < 0.001$ , this being the lowest significance level attainable with 999 permutations). In fact, many effects were sufficiently large so that they would be significant at virtually any nominated significance level, there being a very large number of possible permutations available for this design, reflecting the good levels of replication. The mean square (MS) column shows that, whether the analysis is based on dominant abundances or on presence/absence structure, the primary difference between samples was due to sediment type at the 2 different sites, the response over time also being markedly different at the 2 sites (i.e. large  $Se \times Ti$  interaction). In addition to sediment and exposure period effects, there were clearly demonstrated responses to the different pH exposure levels, which again differed with sediment type and exposure time (significant interactions; Table 3). Even the 3-way interaction was significant, primarily reflecting the large effect of the 20 wk exposure on the low pH (5.6) group ( $Ti \times pH$  2-way interaction). This effect was more pronounced in the sandy than in the muddy sediment ( $Se \times Ti \times pH$  3-way interaction).

The presence of interactions for every factor and the dominant role of the sediment (site) term in the PERMANOVA require that interpretation be based on more piecemeal analyses, i.e. first separating out the 2 sediment types. Two-way crossed ANOSIM results (Table 4) allow scaling of the magnitude of these effects using the ANOSIM R statistic which, unlike the

$F$  statistic, takes values over a fixed and interpretable range, from no separation ( $R \approx 0$ ) to perfect separation of the communities ( $R = 1$ ). For both transformed and untransformed data, ANOSIM show that, averaged over time, changes in seawater pH had marked, although not overwhelming, effects on the community. The significant interaction of the pH effect with that of sediment type, as seen in the PERMANOVA (Table 3),

Table 3. Macrofaunal community PERMANOVA (permutational multivariate ANOVA) analyses of 3 crossed, fixed factors; Se: sediment type (2 sites), Ti: Time of exposure (2 and 20 wk), pH: pH levels (control, 7.3, 6.5, 5.6); for untransformed counts and presence/absence data. Type I SS is given (= Type III in this case since the design fully balanced). 999 permutations of residuals were carried out (either under reduced or full model fitting, the outcome being the same here). The true  $F$  values for all effects are larger than under any of the permutations, hence significant ( $p < 0.001$ ) in every case

Source	df	SS	MS	Pseudo- $F$	p (perm)
<b>Macrofauna: untransformed</b>					
Se	1	99189	99189	90.2	0.001
Ti	1	9890	9890	9.0	0.001
pH	3	14632	4877	4.4	0.001
$Se \times Ti$	1	16575	16575	15.1	0.001
$Se \times pH$	3	10228	3409	3.1	0.001
$Ti \times pH$	3	13148	4383	4.0	0.001
$Se \times Ti \times pH$	3	12407	4136	3.8	0.001
Res	64	70365	1100		
Total	79	246430			
<b>Macrofauna: presence/absence</b>					
Se	1	68721	68721	74.8	0.001
Ti	1	9517	9517	10.4	0.001
pH	3	19759	6586	7.2	0.001
$Se \times Ti$	1	8878	8878	9.7	0.001
$Se \times pH$	3	10617	3539	3.9	0.001
$Ti \times pH$	3	13715	4572	5.0	0.001
$Se \times Ti \times pH$	3	10294	3431	3.7	0.001
Res	64	58797	919		
Total	79	200300			

Table 4. Global R values from 2-way ANOSIM (analysis of similarities) indicating changes in macrofaunal and meiofaunal community structure in response to pH (across all time groups) and time (across all pH groups). All values were significant

	pH		Time	
	Mud	Sand	Mud	Sand
<b>Macrofauna</b>				
Untransformed	0.349	0.502	0.678	0.785
Presence/absence	0.508	0.482	0.545	0.607
<b>Meiofauna</b>				
Untransformed	0.378	0.244	0.433	0.528
Presence/absence	0.255	0.126	0.299	0.299

appeared relatively weak, given the modest difference in average pH-effect R values (Table 4) for the 2 sediment types (0.35 vs. 0.50 for mud and sand, untransformed data, and 0.51 vs. 0.48 for mud and sand, presence/absence data). However, the time-averaged effects represented by the 2-way ANOSIM do not tell the whole story if there are time  $\times$  pH interactions, as the PERMANOVA results have demonstrated. In other words, effects of severe pH drops over a prolonged period (20 wk) are more substantial than would be expected from simply adding the effects of severe pH drops over a short period (2 wk) to the effects of more modest pH drops over a prolonged period (i.e. the meaning of time  $\times$  pH interaction). It is thus necessary to look at the R values from 1-way ANOSIM tests, breaking down comparisons of pairs of pH levels separately for each combination of sediment type and exposure period (Table 5).

The pairwise ANOSIM R values show a very clear pattern. There were more significant and larger differences after 20 wk than were evident after 2 wk. Although significant differences were seen for both untransformed and presence/absence data, the strongest effects were seen when using presence/absence data. This indicates that the impacts of changing seawater pH on the richness and identity of the full suite of species present would be stronger than on the abundances of numerically dominant species (more likely to be reflected in evenness measures). Another clear feature is the strong distinction between the effects of pH 5.6 and all other pH levels by the end of

Table 6. Adjusted R<sup>2</sup> values for the 4 fitted relationships of macrofaunal species diversity ( $\sqrt{S}$ ), abundance ( $\log_{10}NI$ ) and evenness ( $J'$ ) with seawater pH. Bold values represent a rejection of  $H_0$  ( $p < 0.05$ ) thereby indicating where the constrained model did not fit the data as well as the fully unconstrained (ANOVA) model. The best model was identified as the least constrained model that was not significantly different from the full ANOVA model

	2 wk			20 wk		
	$\sqrt{S}$	$\log_{10}NI$	$J'$	$\sqrt{S}$	$\log_{10}NI$	$J'$
<b>Mud</b>						
None	0	0	0	<b>0</b>	<b>0</b>	<b>0</b>
Linear	20.9	13.0	0	<b>74.7</b>	<b>54.7</b>	41.6
Quadratic	18.0	7.9	0	<b>90.2</b>	69.0	48.5
ANOVA	20.4	18.16	0	92.9	70.3	47.48
Best model	None	None	None	ANOVA	Quadratic	Linear
<b>Sand</b>						
None	<b>0</b>	<b>0</b>	0	<b>0</b>	<b>0</b>	0
Linear	61.2	<b>66.1</b>	11.6	<b>59.4</b>	<b>63.4</b>	3.4
Quadratic	60.5	<b>80.4</b>	15.9	<b>84.1</b>	81.9	14.4
ANOVA	62.7	84.5	20.5	89.6	84.72	12.4
Best model	Linear	ANOVA	None	ANOVA	Quadratic	None

20 wk (R values close to 1), but more moderate differences between higher pH levels which were also in increasing sequence. This shows that the greater the change in seawater pH, the larger the change in macrofaunal community structure. A broadly similar pattern was seen for sandy and muddy sediments except that for samples from the sandy site, pronounced effects were seen at 2 as well as at 20 wk (hence the large Ti  $\times$  Se interaction term), with high R values for comparisons with the pH 5.6 group.

In the sandy sediment, there were significant relationships between increasing seawater acidity and both species richness and total abundance (Fig. 3, Table 6).

Table 5. R values from 1-way ANOSIM indicating changes in macrofaunal and meiofaunal community structure in response to pH. Significant differences ( $p < 0.05$ ) are indicated in bold. P/A: presence/absence, C: control

	Macrofauna				Meiofauna			
	Untransformed		P/A		Untransformed		P/A	
	2 wk	20 wk	2 wk	20 wk	2 wk	20 wk	2 wk	20 wk
<b>Mud</b>								
C vs. 7.3	0.080	-0.024	0.008	-0.160	<b>0.312</b>	0.032	0.152	-0.222
C vs. 6.5	0.140	0.156	<b>0.332</b>	<b>0.488</b>	0.252	<b>0.396</b>	<b>0.374</b>	0.016
C vs. 5.6	0.196	<b>1.00</b>	<b>0.552</b>	<b>1.000</b>	-0.144	<b>0.968</b>	-0.048	<b>0.704</b>
7.3 vs. 6.5	0.216	0.060	0.190	<b>0.240</b>	0.188	0.040	<b>0.258</b>	<b>0.800</b>
7.3 vs. 5.6	<b>0.196</b>	<b>0.992</b>	<b>0.414</b>	<b>1.000</b>	0.220	<b>0.964</b>	0.042	<b>0.780</b>
6.5 vs. 5.6	0.100	<b>0.840</b>	<b>0.404</b>	<b>1.000</b>	<b>0.412</b>	<b>0.976</b>	0.024	<b>0.768</b>
<b>Sand</b>								
C vs. 7.3	0.032	0.002	-0.104	-0.02	0.048	<b>0.268</b>	0.036	0.124
C vs. 6.5	0.208	<b>0.298</b>	0.188	<b>0.262</b>	0.292	-0.176	-0.096	-0.022
C vs. 5.6	<b>0.928</b>	<b>0.992</b>	<b>0.660</b>	<b>1.000</b>	0.096	<b>0.420</b>	-0.160	<b>0.298</b>
7.3 vs. 6.5	0.164	0.144	0.014	<b>0.448</b>	0.156	0.172	-0.011	0.198
7.3 vs. 5.6	<b>0.774</b>	<b>0.940</b>	<b>0.494</b>	<b>1.000</b>	0.228	<b>0.780</b>	0.118	<b>0.868</b>
6.5 vs. 5.6	<b>0.920</b>	<b>0.948</b>	<b>0.496</b>	<b>1.000</b>	0.064	<b>0.472</b>	-0.200	<b>0.724</b>

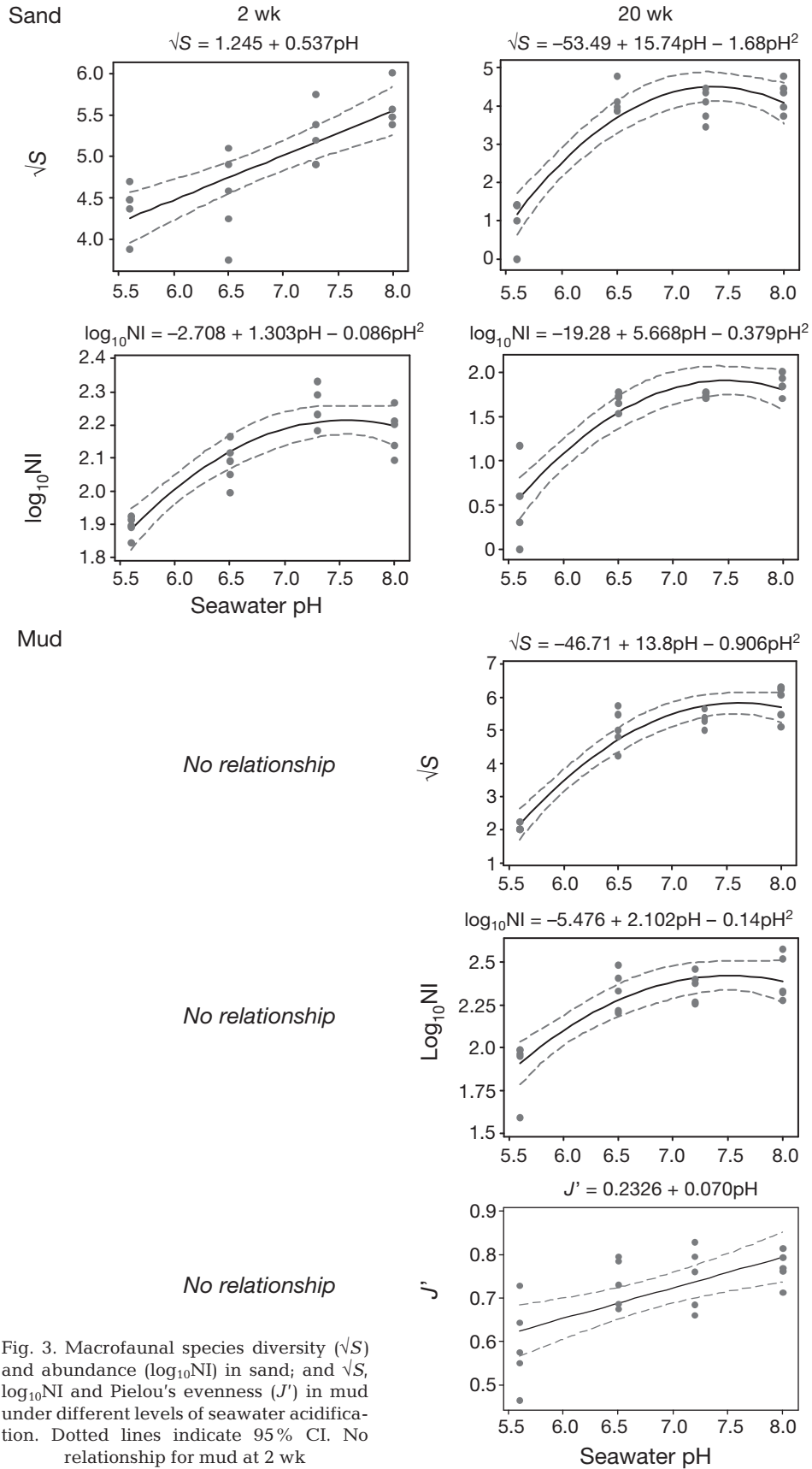


Fig. 3. Macrofaunal species diversity ( $\sqrt{S}$ ) and abundance ( $\log_{10}NI$ ) in sand; and  $\sqrt{S}$ ,  $\log_{10}NI$  and Pielou's evenness ( $J'$ ) in mud under different levels of seawater acidification. Dotted lines indicate 95% CI. No relationship for mud at 2 wk



Relationships were evident both after 2 wk exposure and at the end of the experiment. Seawater acidification had no impact on measurements of Pielou's evenness in the sandy sediment, even after 20 wk.

In the muddy sediment, significant relationships between increasing seawater acidity and both species richness and total number of individuals were also evident after 20 wk (Fig. 3, Table 6). However, unlike the sandy sediment, where significant relationships were observed after 2 wk exposure, the 2 wk data for muddy sediment showed no relationship with seawater acidity. The muddy sediment demonstrated a significant relationship between seawater acidity and Pielou's  $j'$  evenness index after 20 wk but with much lower 'variance explained' ( $R^2$ ) than for the relationships of species richness and total numbers with seawater pH.

#### Impact of seawater acidification on nematode community structure and diversity

PERMANOVA results (Table 7) show that for untransformed nematode abundance data, all main effects, 2-way and 3-way interactions were significant. When the data were transformed (presence/absence),

Table 7. Nematode community PERMANOVA (permutational multivariate ANOVA) analyses of 3 crossed, fixed factors, Se: sediment type (2 sites), Ti: Time of exposure (2 and 20 wk), pH: pH levels (control, 7.3, 6.5, 5.6), for untransformed counts and presence/absence data. Type I SS is given (= Type III in this case since the design fully balanced). 999 permutations of residuals were carried out (either under reduced or full model fitting, the outcome being the same here)

Source	df	SS	MS	Pseudo- $F$	p (perm)
<b>Nematodes: untransformed</b>					
Se	1	56276	56276	47.5	0.001
Ti	1	11640	11640	9.8	0.001
pH	3	13048	4349	3.7	0.001
Se $\times$ Ti	1	8965	8695	7.6	0.001
Se $\times$ pH	3	6356	2119	1.8	0.002
Ti $\times$ pH	3	11517	3839	3.2	0.001
Se $\times$ Ti $\times$ pH	3	9028	3009	2.5	0.001
Res	64	75902	1186		
Total	79	192730			
<b>Nematodes: presence/absence</b>					
Se	1	26060	26060	25.8	0.001
Ti	1	3650	3650	3.5	0.001
pH	3	10494	3498	3.6	0.001
Se $\times$ Ti	1	2815	2815	2.8	0.001
Se $\times$ pH	3	2997	999	1.0	0.511
Ti $\times$ pH	3	9004	3001	3.0	0.001
Se $\times$ Ti $\times$ pH	3	3931	1312	1.3	0.086
Res	64	64639	1010		
Total	79	123600			

the main effects and 2 of the 2-way interactions remained significant, although the 3-way interaction and the interaction between sediment type and pH became non-significant. As with the macrofauna data, the MS column in Table 7 shows that, irrespective of analysis method (presence/absence or dominant abundance), the primary difference in nematode community structure between samples was due to sediment type. Although smaller than was observed for macrofauna, nematode community responses to different exposure periods and pH levels were also clearly demonstrated.

The presence of many significant interactions and the dominant role of the sediment (site) term in the PERMANOVA again require that interpretation be based on more piecemeal analyses. Two-way crossed ANOSIM results (Table 4) show that changes in seawater pH had significant effects on both the relative abundance of numerically dominant species (untransformed data) and the presence or absence of nematode species, although with generally smaller community changes (lower ANOSIM  $R$ ) than in the corresponding macrofauna data. These significant effects were evident in both muddy and sandy sediments.

As in macrofauna, 1-way ANOSIM results (Table 5) show that most of the significant differences occurred after 20 rather than after 2 wk for the nematode community data. The ANOSIM  $R$  values (community separations) are usually substantially larger for the longer exposure period. As seen in the 2-way ANOSIM, effects on meiofauna exposed to different pH values (at virtually all levels) for 20 wk were less pronounced than corresponding effects on macrofauna. Once again, both the abundance of numerically dominant species as well as the pattern of rarer species were significantly impacted by seawater acidification.

Significant relationships were also observed between seawater pH and nematode species richness, total abundance and Pielou's evenness, but again only after 20 wk exposure (Fig. 4, Table 8).

#### Impact of seawater acidification on nutrient flux

In the sandy sediment, significant relationships were observed between seawater pH and nutrient flux rates for nitrite, nitrate, ammonium, and silicate (Fig. 5, Table 9). These relationships were detectable after 2 and 20 wk. Decreasing seawater pH decreased the release of nitrate, nitrite and silicate from the sediment and increased the release of ammonium (Fig. 5). Seawater pH had no impact on the flux of phosphate (Table 9).

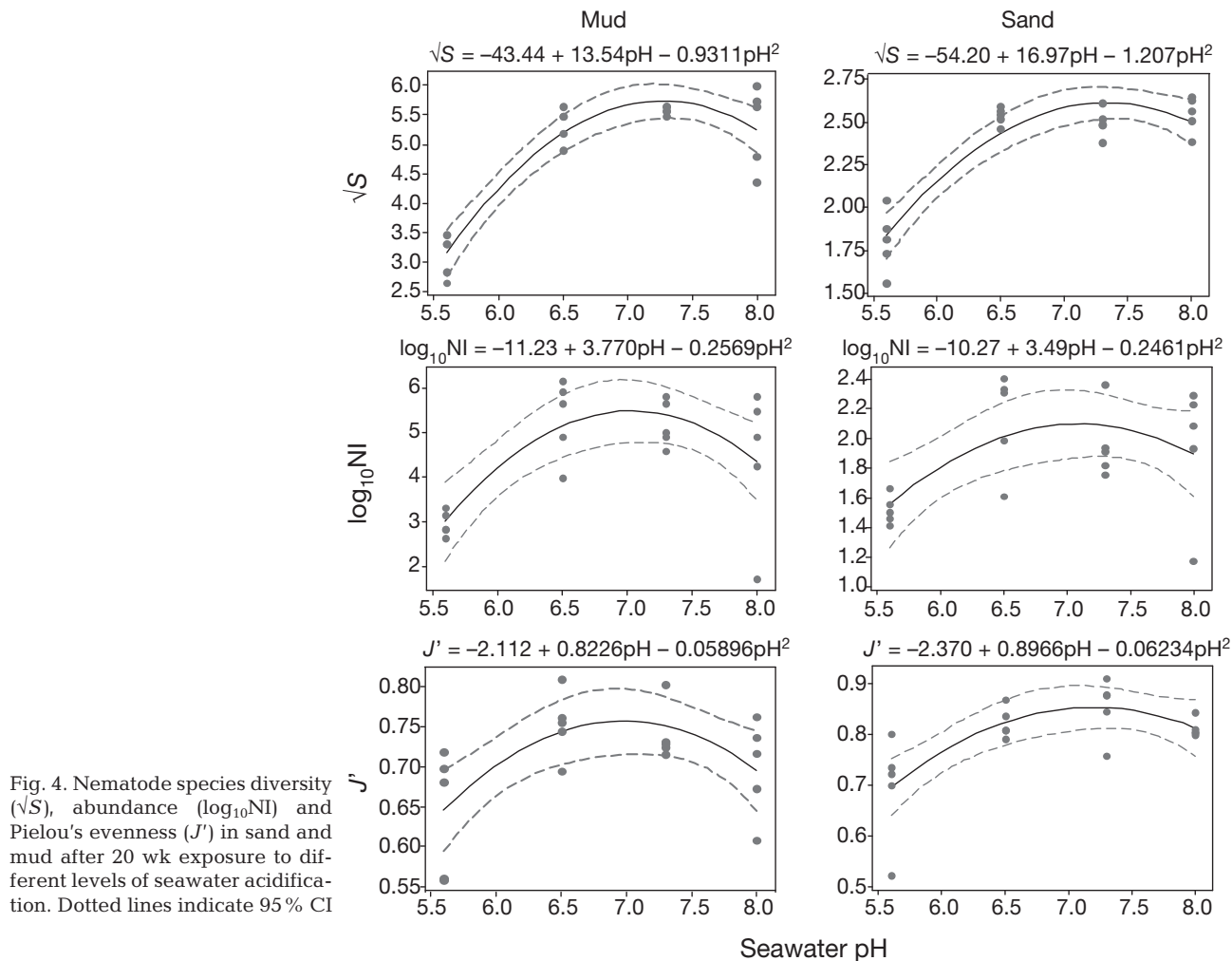


Fig. 4. Nematode species diversity ( $\sqrt{S}$ ), abundance ( $\log_{10}NI$ ) and Pielou's evenness ( $J'$ ) in sand and mud after 20 wk exposure to different levels of seawater acidification. Dotted lines indicate 95% CI

Table 8. Adjusted  $R^2$  values for the 4 fitted relationships of nematode species diversity ( $\sqrt{S}$ ), abundance ( $\log_{10}NI$ ) and evenness ( $J'$ ) to seawater pH. Bold values represent a rejection of the  $H_0$  ( $p < 0.05$ ) model, thereby indicating where the constrained model did not fit the data as well as the fully unconstrained (ANOVA) model ( $H_1$ ). The best model was identified as the least constrained model that was not significantly different from the full ANOVA model

	2 wk			20 wk		
	$\sqrt{S}$	$\log_{10}NI$	$J'$	$\sqrt{S}$	$\log_{10}NI$	$J'$
<b>Mud</b>						
None	<b>0</b>	0	0	<b>0</b>	<b>0</b>	<b>0</b>
Linear	22.8	0	0	<b>53.2</b>	<b>56.8</b>	<b>3.7</b>
Quadratic	28.9	21.8	0	84.9	<b>81.9</b>	33.9
ANOVA	26.31	17.5	7.0	85.28	88.3	31.21
Best model	Linear	None	None	Quadratic	ANOVA	Quadratic
<b>Sand</b>						
None	0	0	0	<b>0</b>	<b>0</b>	<b>0</b>
Linear	0	0	0	<b>12.1</b>	9.0	<b>25.9</b>
Quadratic	0	0	7.6	47.2	25.4	47.7
ANOVA	0	0	6.3	45.4	28.7	44.4
Best model	None	None	None	Quadratic	Linear	Quadratic

In the muddy sediment, there were no significant effects of changing seawater pH on the fluxes of nitrate, nitrite, silicate or phosphate (Table 9). The only significant effect was an increase in ammonium efflux in low pH treatments after 2 wk (Fig. 6, Table 9). This effect was not observed after 20 wk (Table 9).

Both mud and sand had similar flux rates in the control treatments for ammonium, silicate and phosphate. However, the average rate of nitrite release in the control treatments (2 and 20 wk combined) was higher in the sandy (9.98 ± 3.97  $\mu\text{mol m}^{-2} \text{d}^{-1}$ ) than in the muddy sediment (3.46 ± 1.69  $\mu\text{mol m}^{-2} \text{d}^{-1}$ ). Also, the sandy sediment was a strong source of nitrate (338.2 ± 122.1  $\mu\text{mol m}^{-2} \text{d}^{-1}$ ) while the muddy sediment was a slight sink for nitrate (−18.1 ± 48.9  $\mu\text{mol m}^{-2} \text{d}^{-1}$ ). This implies that the 2 sediment types differed in their relative rates of nitrification and denitrification.

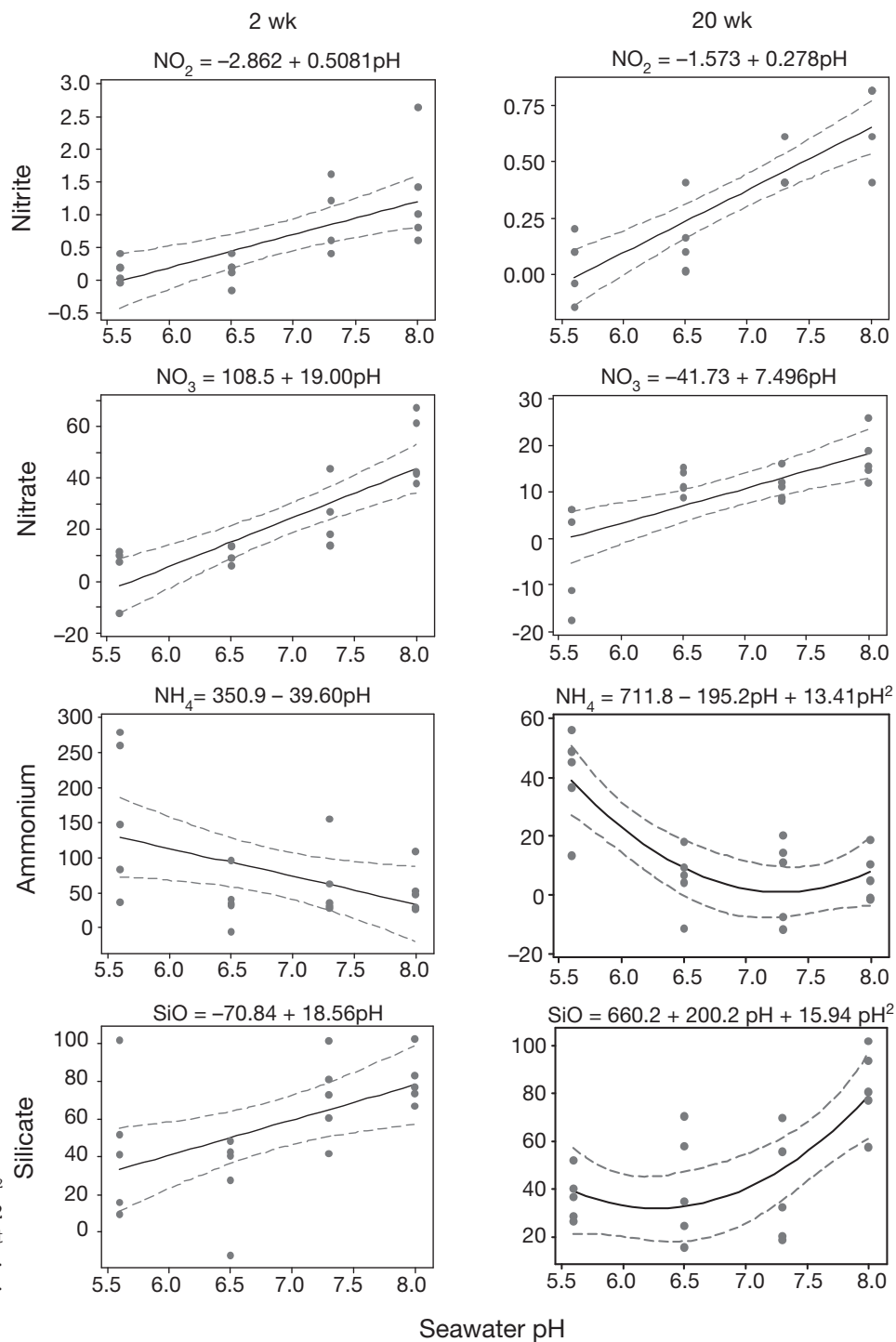


Fig. 5. Nutrient flux (µmol m<sup>-2</sup> d<sup>-1</sup>) in a sandy sediment after 2 and 20 wk exposure to different levels of seawater acidification. Positive flux indicates an efflux. Dotted lines indicate 95% CI

### Impact of seawater acidification on sediment carbon and nitrogen concentrations

Changes in seawater pH had no impact on the carbon or nitrogen content of both sediment types after either 2 or 20 wk exposure. The only significant changes in sediment carbon or nitrogen were seen as a

reduction in both organic carbon ( $p < 0.05$ ) and organic nitrogen ( $p < 0.10$ ) between weeks 2 and 20 across all pH treatments. However, the actual changes were small, with the average percentage of organic carbon declining from  $0.53 \pm 0.13$  to  $0.46 \pm 0.08\%$  and that of organic nitrogen declining from  $0.039 \pm 0.012$  to  $0.034 \pm 0.006\%$ .

Table 9. Adjusted  $R^2$  values for the 4 potential relationships between seawater pH and nutrient fluxes. Bold values represent a rejection of the  $H_0$  thereby indicating where the constrained model fitted the data as well as the fully unconstrained (ANOVA) model ( $H_1$ ). The best model was identified as the least constrained model that was not significantly different from the ANOVA model. (No = no relationship, Lin = linear model, Quad = quadratic model, An = ANOVA model)

	2 wk					20 wk				
	NH <sub>4</sub>	NO <sub>2</sub>	NO <sub>3</sub>	SiO	PO <sub>4</sub>	NH <sub>4</sub>	NO <sub>2</sub>	NO <sub>3</sub>	SiO	PO <sub>4</sub>
<b>Mud</b>										
None	<b>0</b>	0	0	0	0	0	0	0	0	0
Linear	<b>62.3</b>	0	0	3.6	0	14.4	15.1	8.2	0	2.9
Quadratic	<b>73.1</b>	0	0	1.1	0	24.2	10.5	2.1	0	2.7
ANOVA	80.7	0	16.1	12.4	0	19.6	14.2	14.6	8.5	0
Best model	An	No	No	No	No	No	No	No	No	No
<b>Sand</b>										
None	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	0	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	0
Linear	17.9	43.8	66.0	25.2	0	<b>36.1</b>	73.5	48.3	<b>29.2</b>	0
Quadratic	28.1	46.0	74.1	27.7	0	55.5	75.8	50.2	42.9	0
ANOVA	30.2	45.0	72.8	34.3	0	56.0	75.3	54.4	47.0	0
Best model	Lin	Lin	Lin	Lin	No	Quad	Lin	Lin	Quad	No

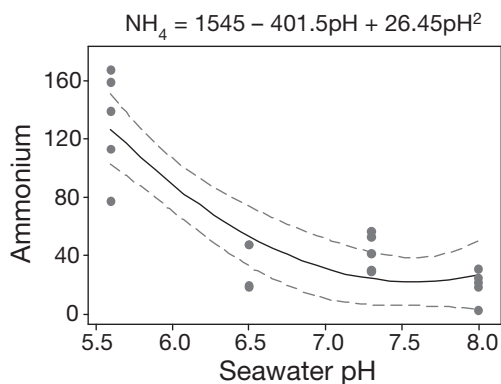


Fig. 6. Ammonium flux ( $\mu\text{mol m}^{-2} \text{d}^{-1}$ ) in a muddy sediment after 2 wk exposure to different levels of seawater acidification. Positive flux indicates an efflux. Dotted lines indicate 95% CI

## DISCUSSION

This is the most extensive experiment, to date, conducted to examine the impact of changing seawater pH on the fauna and function of benthic sediments. The study has demonstrated that a change in seawater pH has the potential to affect the diversity and abundance of benthic organisms and alter the flux of key nutrients across the sediment–water interface, particularly in permeable sediments. This study also highlights the variability of responses between sandy and muddy sediment types and between macrofauna and meiofauna.

## Impact of seawater acidification on macrofaunal community structure and diversity

Significant impacts of seawater acidification on macrofaunal community structure and diversity were observed in both sediment types used in the current study. However, it is clear that impacts on the sandy sediment fauna were greater and occurred more quickly than those on the muddy sediment fauna. The reasons for this are unknown but it is possible that, as muddy sediments have generally lower oxygen availability and higher  $\text{CO}_2$  levels than sandy sediments, mud fauna may be acclimated to a more hypercapnic environment than sand fauna. The differences could also be due to potential variations in the geochemical buffering capacities of the 2 sediment types. However, this seems

unlikely in the current experiment as Fig. 2 demonstrates very little difference in the impacts of seawater acidification on the pH profiles between the 2 different sediments.

The ability of an organism to survive periods of hypercapnia is linked to metabolic and physiological mechanisms. Variations among taxa in the extent of their ability to buffer extracellular pH are likely to lead to communities consisting of organisms with widely differing tolerances to elevated  $\text{CO}_2$  (Widdicombe & Spicer 2008). While echinoderms show very little compensation for hypercapnia-related disturbance in extracellular acid–base balance (Spicer et al. 1988, Spicer 1995), other phyla employ active transport mechanisms. Crustaceans, for example, use the coupled transfer of acid–base equivalents to ion exchange as the principal mechanism of acid–base regulation (Wheatly & Henry 1992). While reliance on calcium carbonate structures to temporarily buffer extracellular acidosis (e.g. in echinoderms and molluscs) is sustainable during short-term exposures (days to weeks), continual exposure will eventually exhaust the available carbonate. This will have implications for skeletal strength (Seibel & Walsh, 2003), calcification (Gazeau et al. 2007) and growth (Berge et al. 2006). Thus, providing the energetic costs of active transport mechanisms do not become too great, organisms that adopt this mechanism should be more tolerant to hypercapnia than those that do not.

It has been suggested that some marine organisms can survive periods of energy limitation (oxygen or food) by suppressing their metabolism (Guppy & With-

ers 1999). Reduced pH and/or hypercapnia can reportedly trigger this response partly by shutting down expensive cellular processes such as protein synthesis (Guppy & Withers 1999). This mechanism, while reversible and effective for surviving short periods of hypercapnia, will result in reduced growth and reproduction during longer exposure periods. However, experiments that have demonstrated metabolic suppression in response to hypercapnia have all been conducted over short periods of time, mostly <5 d. In longer-term (several weeks or more) experiments (e.g. Widdicombe & Needham 2007), data indicate that metabolic suppression is not an important mechanism in surviving long-term exposure to elevated CO<sub>2</sub>.

While elevated CO<sub>2</sub> may directly affect the survival of marine species by impacting extracellular acid–base regulation, one should also consider that the structure and diversity of communities are strongly influenced by interactions between individual organisms (e.g. predator–prey relationships, competition). While a number of experiments on individual species effects have now been conducted, the impacts on organism interactions are as yet unknown. The only published study to date that has examined the impact of high CO<sub>2</sub> on animal interactions (Bibby et al. 2007) showed significant differences in the behaviour of a prey species (in response to the threat of predation) as a result of hypercapnic exposure. While the changes in community structure observed in the current study are most likely due to direct effects on individual survival, the possibility of secondary effects, caused by changes in species interactions, exists when observing complex, multi-species assemblages.

#### **Impact of seawater acidification on nematode community structure and diversity**

The current study has shown that increased seawater acidity had a significant impact on the structure and diversity of nematode communities in both muddy and sandy sediments. However, examination of the size of the main effects present in the relative PERMANOVA analyses (Tables 3 & 7) shows that these impacts were less severe than those observed for the macrofaunal communities. This apparently greater short-term tolerance of nematodes to hypercapnia could be due to their possession of an impermeable proteinaceous cuticle (Brusca & Brusca 1990). Diffusion of CO<sub>2</sub> through external membranes will quickly equilibrate the CO<sub>2</sub> tensions and pH of extracellular fluids with ambient seawater concentrations. With their thicker cuticle and internal positive pressure, nematodes may therefore be temporarily buffered more effectively than other taxa (Barry et al. 2004). How-

ever, persistently high CO<sub>2</sub> will eventually result in extracellular acidosis.

The current study is only the second study to report the response of shallow-water nematodes to seawater acidification. The first study (Dashfield et al. 2008) used only one acidified treatment (pH 7.5) and found nematodes to be unaffected by high CO<sub>2</sub> after days of exposure at this treatment level. However, they observed that a significant change in nematode community structure due to acidification induced changes in the survival and activity of a large bioturbating echinoderm, *Echinocardium cordatum*. All other previous studies have examined deep-water nematodes, thus, in making comparisons between the current and these previous studies, it should be considered that deepwater organisms may be more susceptible to hypercapnia than those of shallow waters (Seibel & Walsh 2003, Pane & Barry 2007). Ishida et al. (2005) reported that nematodes can withstand short-term exposure to extremely high levels of CO<sub>2</sub> and found no significant reduction in nematode abundance after nearly 16 d exposure to CO<sub>2</sub> levels of 5000 ppm (~pH 7). Takeuchi et al. (1997) also found no significant impact on nematode abundance after 7 d exposure to pH 7 or 6.2. It appears, from both the current and previous studies, that nematodes are likely to be able to withstand short-term exposure to even severe seawater acidification and are likely to be less affected by the leakage of CO<sub>2</sub> from geological storage than are macrofauna.

#### **Impact of seawater acidification on nutrient flux**

In a previous mesocosm experiment, Widdicombe & Needham (2007) suggested that decreasing seawater pH could affect sediment nitrogen cycling by impacting the process of nitrification. Their conclusions were based on observed changes in the sediment fluxes of nitrite, nitrate and ammonium. In the sandy sediment, the current study observed similar significant changes in the fluxes of these 3 nutrients, lending support to the findings of Widdicombe & Needham (2007).

The only study to date that measured the impact of seawater acidification on nitrification in the open ocean (Huesemann et al. 2002) demonstrated that rates of ammonium oxidation to nitrite or nitrate (nitrification) were reduced by ~50% at pH 7, by >90% at pH 6.5 and were completely inhibited at pH 6. If rates of nitrification had been reduced in the current study, it would explain the reduction in the release of both nitrite and nitrate and the increase in the release of ammonium with decreased seawater pH in the sandy sediment. It should be noted that Huesemann et al. (2002) studied a pelagic system and that no direct evi-

dence currently exists for acid-induced reductions in nitrification rates in sediment systems. However, the current study, together with that of Widdicombe & Needham (2007), offers strong supporting evidence that reduced sediment nitrification occurred in response to increased seawater acidification.

The current study also demonstrated a significant increase in ammonium release in response to acidified seawater at pH 5.6. Widdicombe & Needham (2007) observed a similar change in ammonium flux and evidence from the current study provides further support to their suggestions concerning the role of anaerobic ammonium oxidation (anammox) in nitrogen cycling and the impact of acidification on this process. Anammox is a significant process in the conversion of fixed nitrogen into atmospheric nitrogen ( $N_2$ ) gas (Op den Camp et al. 2006) and may account for nearly 80% of the total  $N_2$  production in some coastal sediments (Engstrom et al. 2005). Therefore, if more ammonium oxidation occurs via the anammox process than through nitrification under acidic conditions, as Widdicombe & Needham (2007) suggested, increases in ammonium release in response to seawater acidification would not be as great as would be expected from the proposed reduction in nitrification. While this hypothesis could explain the observed patterns in ammonium flux, it relies on the assumption that the microbial organisms responsible for anammox are more tolerant to pH changes than those responsible for nitrification. As Widdicombe & Needham (2007) admitted, this assumption is highly speculative and remains to be tested.

The different relationships between seawater pH and ammonium flux, seen at 2 and 20 wk in the sandy sediment, may be explained by considering the effects of acidification on the survival and activity of *Echinocardium cordatum*. After 2 wk, it was observed that all the *E. cordatum* in pH treatments 5.6 and 6.5 had come to the surface and died. All urchins in the control and pH 7.3 treatments were alive, deeply buried and healthy, as determined by histological analysis (S. Widdicombe pers. obs.). After 20 wk exposure, all urchins in the control and pH 7.3 treatments were still alive. However, those in the pH 7.3 treatments were found closer to the sediment surface than those in the control treatments. Additional histological analysis of the urchins in the pH 7.3 treatments showed them to be in very poor health compared with those in the controls.

Apart from an initial impact on ammonium flux after 2 wk, no pH-induced changes in nutrient flux were seen in the muddy sediment treatments. For nitrogen fluxes, this lack of response may have been caused by differences in the nitrification–denitrification coupling between the 2 sediment types. It is considered that, due to oxygen availability, sediment nitrification rates

are higher in sandy sediments, compared to muddy sediments. Consequently, the nitrate required to fuel denitrification in sandy sediments is largely generated within the sediment and the net nitrate flux is often an efflux. In contrast, nitrification in muddy sediments does not produce enough nitrate to fully fuel denitrification and additional nitrate is taken from the overlying water, resulting in an influx. This situation was seen in the current study with the average nitrate flux (averaged across 2 and 20 wk) in the sandy sediment being  $33.8 \pm 3.8 \mu\text{mol m}^{-2} \text{d}^{-1}$  and that in the muddy sediments being  $-1.8 \pm 4.9 \mu\text{mol m}^{-2} \text{d}^{-1}$ . Consequently, if the main impact of seawater acidification on nitrogen cycling is a reduction in nitrification, a stronger response would be expected in sediments where nitrification is likely to be most important.

The current study indicated a significant impact of seawater acidification on the release of silicate in the sandy sediment. This contrasts with the results of Widdicombe & Needham (2007) who found no such effect of acidification on silicate flux. However, they demonstrated that the presence of a deposit feeding polychaete, *Nereis virens*, increased the release of silicate probably through the excretion of silicate-rich waste products. This is highly relevant to the current study because the sandy sediment treatments used here also contained a large deposit feeding organism, the burrowing echinoderm *Echinocardium cordatum*. This species is also known to increase the release of silicate in sandy sediments (Townsend 2006). Statistical analysis reveals that after 2 wk of exposure, the relationship between seawater pH and silicate flux was weakly linear. After 20 wk exposure, a quadratic relationship between seawater pH and silicate flux had been established, with the greatest changes occurring between the controls and pH 7.3.

The lack of acidification effects observed for phosphate flux would be further evidence to support the assumption that the changes in the silicate flux were driven biologically rather than chemically. As the anions  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{3-}$  adsorb onto the same components in the sediment and have common chemical reactions (Hartikainen et al. 1996), the lack of any pH effects on the flux of phosphate would indicate that the impact of pH on silicate flux was not due to any changes in the oxic condition of surface sediments. It is therefore most likely that the effect of seawater acidification on silicate flux occurred as a result of the impacts of acidification on a key functional species rather than as a direct impact on sediment chemistry.

As the impact of seawater acidification on silicate flux in the sandy sediment appeared to be a result of the impact on the bioturbating urchin *Echinocardium cordatum*, a response in the muddy sediment could only be expected if this sediment contained a function-

ally similar organism. As the mud did not contain such an organism, it is not surprising that no impact on silicate was observed in this sediment.

### Response of marine benthos to leakage from geological CO<sub>2</sub> storage

CO<sub>2</sub> released from a sub-seabed geological store has the potential to considerably reduce seawater pH in the locality of the leak (Blackford & Gilbert 2007). The results of the current study have demonstrated that such a leakage would cause a significant change in community structure and loss of benthic biodiversity. As already discussed, these community changes would be caused by the different tolerances of benthic organisms to high CO<sub>2</sub>. However, to provide accurate predictions as to the order in which species are likely to be lost from a leak site, more experiments are required to identify the mechanisms by which high CO<sub>2</sub> impacts the physiology and activity of benthic organisms.

A rather surprising outcome of the current study was that even in the extremely acidified treatments (pH 5.6), some species (primarily capitellid worms and nematodes) persisted after 20 wk. The persistence of these organisms will have significant, positive implications for the recovery of benthic sediments after a CO<sub>2</sub> leak. Previous studies using field experiments and observations have shown that the addition of bioturbating species, particularly capitellid polychaetes, can 'recondition' organically polluted sediments, thereby accelerating the natural recovery process (Chareonpanich et al. 1994, Ueda et al. 1994).

While significant impacts of seawater acidification were observed in both sediment types used in the current study, it is clear that impacts on sandy sediment fauna and nutrient fluxes were larger and occurred more quickly than those on muddy sediment fauna and nutrient fluxes. Although the precise mechanisms by which these results occurred are unknown, as discussed earlier, variations in response between the 2 sediment types will have implications when considering the location of sites for the storage of CO<sub>2</sub>. In addition, these results highlight the importance of capturing sediment variability in models intended to predict the impact of CCS leakage on marine ecosystems (e.g. Blackford & Gilbert 2007). Underground geological storage of CO<sub>2</sub> is an important approach to mitigate the high atmospheric CO<sub>2</sub> concentration predicted in the future. It is assumed that the probability of leakage from a single site is low. However, widespread use of geological storage of CO<sub>2</sub> will obviously increase the overall probability of leak occurrence. Avoiding geological storage of CO<sub>2</sub> without implementing alternative measures to reduce marine acidification may also

lead to adverse effects on benthic organisms. Despite the observations of significant effects of CO<sub>2</sub> on benthic organisms, this argument should not be used as a major issue to prevent geological storage of CO<sub>2</sub>. The environmental repercussions of not sequestering CO<sub>2</sub> are still greater than those created by potential leaks.

A final observation from the current study that has interesting implications for sub-seabed storage of CO<sub>2</sub> was the response of the burrowing urchins *Echinocardium cordatum* to seawater acidification. One major concern of regulators and environmental managers is how to detect and locate leaks of CO<sub>2</sub> should they occur. Areas that are potentially at risk from a leak are likely to be vast. It is therefore impractical to set up a comprehensive network of pH or pCO<sub>2</sub> sensors to monitor the whole area. Instead, it may be possible to employ biological monitoring using towed underwater video to assess site status. During the current study, *E. cordatum* were seen to come to the sediment surface under high CO<sub>2</sub> conditions. This is a common response in burrowing organisms when oxygen levels are low (Rosenberg et al. 1991). While oxygen levels were not low in the current study, it is likely that the response was triggered by hypercapnia. This surfacing response could thus be a useful visual indication that a leak is present.

### Impact of ocean acidification on marine benthos

When attempting to determine the likely impacts of ocean acidification on marine organisms, concerns exist about the validity of experiments which use pH levels that are lower than those predicted under realistically high CO<sub>2</sub> scenarios. It is argued that such experiments produce 'shock' responses that are not representative of the temporal scale by which seawater chemistry is actually changing. Many marine organisms have mechanisms, such as reduced metabolic activity or growth, by which they can physiologically compensate for short-term (weeks to months) exposure to hypercapnia and thereby ensure survival (Pörtner et al. 2004). However, such compensatory mechanisms will place an energetic burden on the individual which could be unsustainable in the long term. A recent study by Wood et al. (2008) showed that the burrowing brittlestar *Amphiura filiformis* reabsorbed its own muscle tissue to fuel the physiological mechanisms required to survive periods of hypercapnia during a 40 d exposure to pH 7.6. It is possible, therefore, that due to the relatively short timescale over which they are run, experiments which use more subtle changes in pH (e.g. 0.3 or 0.4 pH units) are in danger of underestimating the long-term impacts of ocean acidification. The value of 'shock' experiments, such as the cur-

rent study, is that they are able to determine the organisms which have the lowest tolerances to hypercapnia and are therefore the ones most likely to be impacted by ocean acidification.

The current study has illustrated that considerable variability in tolerance exists among different organisms that constitute a community and that ocean acidification has the potential to reduce benthic biodiversity. While the current study was limited to the effects on adults, it should be remembered that the early life stages are often the most vulnerable part of an organism's life cycle. This may be particularly true for the many benthic species that have pelagic larvae; the impact of ocean acidification could be hardest felt by juveniles. Recent studies using realistic ocean acidification scenarios have demonstrated significant effects of elevated CO<sub>2</sub> on the development of echinoderm (Kurihara & Shiraiyama 2004) and mollusc (Kurihara et al. 2007) larvae. Even in relatively tolerant organisms such as polychaetes, there is evidence that hypercapnia can significantly slow down larval development at levels of seawater pH predicted for the year 2100 (M. A. Kendall pers. comm.). What are needed now are more studies to determine the metabolic, physiological and ecological mechanisms by which hypercapnia affects survival across a range of taxonomic groups and life stages. With this information, it should be possible to predict the likely impacts of ocean acidification on the viability of marine species and therefore the likely structure and function of future benthic communities.

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#### LITERATURE CITED

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth
- Barnes RSK, Hughes RN (1988) An introduction to marine ecology. Blackwell Scientific Publications, Oxford
- Barry JP, Buck KR, Lovera CF, Kuhn L and others (2004) Effects of direct ocean CO<sub>2</sub> injection on deep-sea meiofauna. *J Oceanogr* 60:759–766
- Berge JA, Bjerkeng B, Schaanning MT, Pettersen O, Øxnevad S (2006) Effects of increased seawater concentrations of CO<sub>2</sub> on growth of the bivalve *Mytilus edulis* L. *Chemosphere* 62:681–687
- Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol Lett* 3: 699–701
- Blackford JC, Gilbert F (2007) pH variability and CO<sub>2</sub> induced acidification in the North Sea. *J Mar Syst* 64:229–241
- Blackford J, Jones N, Proctor R, Holt J, Widdicombe S, Lowe D, Rees A (in press) An initial assessment of the potential environmental impact of CO<sub>2</sub> escape from marine carbon capture and storage systems. *J Power Energy*
- Brewer PG, Riley JP (1965) The automatic determination of nitrate in seawater. *Deep-Sea Res* 12:765–772
- Brusca RC, Brusca GJ (1990) Invertebrates. Sinauer Associates, Sunderland, MA
- Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425:365
- Chareonpanich C, Tsutsumi H, Montani S (1994) Efficiency of the decomposition of organic matter, loaded on the sediment, as a result of the biological activity of *Capitella* sp. I. *Mar Pollut Bull* 28:314–318
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18:117–143
- Clarke KR, Gorley RN (2006) PRIMER v6: User manual/Tutorial. PRIMER-E, Plymouth
- Clarke KR, Green RH (1988) Statistical design and analysis for a 'biological effects' study. *Mar Ecol Prog Ser* 46: 213–226
- Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth
- Dashfield SL, Somerfield PJ, Widdicombe S, Austen MC, Nimmo M (2008) Impacts of ocean acidification and burrowing urchins on within-sediment pH profiles and subtidal nematode communities. *J Exp Mar Biol Ecol* 365: 46–52
- Engstrom P, Dalsgaard T, Hulth S, Aller RC (2005) Anaerobic ammonium oxidation by nitrite (anammox): implications for N<sub>2</sub> production in coastal marine sediments. *Geochim Cosmochim Acta* 69:2057–2065
- Feely RA, Sabine CL, Lee K, Berelson W, Klepas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO<sub>2</sub> on the CaCO<sub>3</sub> system in the oceans. *Science* 305:362–366
- Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive 'acidified' water onto the continental shelf. *Science* 320:1490–1492
- Fenchel TM, Riedl RJ (1970) A new biotic community underneath oxidized layer of marine sand bottoms. *Mar Biol* 7: 255–268
- Gazeau F, Ouiblier C, Jansen JM, Gattuso JP, Middelburg JJ, Heip C (2007) Impact of elevated CO<sub>2</sub> on shellfish calcification. *Geophys Res Lett* 34:L07603
- Gibbins J, Haszeldine S, Holloway S, Pearce J, Oakey J, Shackley S, Turley C (2006) Scope for future CO<sub>2</sub> emission reductions from electricity generation through the deployment of carbon capture and storage technologies. In: Schellnhuber HJ, Cramer W, Nakicenovic N, Wigley T, Yohe G (eds) Avoiding dangerous climate change. Cambridge University Press, p 379–38
- Grasshoff K (1976) Methods of seawater analysis. Verlag Chemie, Weinheim
- Guppy M, Withers P (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev Camb Philos Soc* 74:1–40



- Hartikainen H, Pitkänen M, Kairesalo T, Tuominen L (1996) Co-occurrence and potential chemical competition of phosphorous and silicon in lake sediment. *Water Res* 30:2472–2478
- Haugan PM, Drange H (1996) Effects of CO<sub>2</sub> on the ocean environment. *Energy Convers Manage* 37:1019–1022
- Hawkins DG (2004) No exit: thinking about leakage from geologic carbon storage sites. *Energy* 29:1571–1578
- Holloway S (2005) Underground sequestration of carbon dioxide—a viable greenhouse gas mitigation option. *Energy* 30:2318–2333
- Huesemann MH, Skillman AD, Crecelius EA (2002) The inhibition of marine nitrification by ocean disposal of carbon dioxide. *Mar Pollut Bull* 44:142–148
- Ishida H, Watanabe Y, Fukuhara T, Kaneko S, Furusawa K, Shirayama Y (2005) In situ enclosure experiment using a benthic chamber system to assess the effect of high concentration of CO<sub>2</sub> on deep-sea benthic communities. *J Oceanogr* 61:835–843
- Jia-Zhong Z, Jie C (2002) Automated analysis of nanomolar concentrations of phosphate in natural waters, with liquid waveguide. *Environ Sci Technol* 36:1048–1053
- Kirkwood D (1989) Simultaneous determination of selected nutrients in seawater. International Council for the Exploration of the Sea (ICES), CM 1989/C:29
- Kristensen E (2001) Impact of polychaetes (*Nereis* spp. and *Arenicola marina*) on carbon biogeochemistry in coastal marine sediments. *Geochem Trans* 2:92–104
- Kurihara H, Shirayama Y (2004) Effects of increased atmospheric CO<sub>2</sub> on sea urchin early development. *Mar Ecol Prog Ser* 274:161–169
- Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased pCO<sub>2</sub> on early development of the oyster *Crassostrea gigas*. *Aquat Biol* 1:91–98
- Mantoura RFC, Woodward EMS (1983) Optimization of the indophenol blue method for the automated determination of ammonia in estuarine waters. *Estuar Coast Shelf Sci* 17:219–224
- McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82:290–297
- Miles H, Widdicombe S, Spicer JI, Hall-Spencer J (2006) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar Pollut Bull* 54:89–96
- Op den Camp HJM, Kartal B, Guven D, van Niftrik LAMP and others (2006) Global impact and application of the anaerobic ammonium-oxidizing (anammox) bacteria. *Biochem Soc Trans* 34:174–178
- Pane EF, Barry JP (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Mar Ecol Prog Ser* 334:1–9
- Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO<sub>2</sub> concentrations: lessons from animal physiology and earth history. *J Oceanogr* 60:705–718
- Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society policy document 12/05. The Clyvedon Press, Cardiff
- Rosenberg R, Hellman B, Johansson B (1991) Hypoxic tolerance of marine benthic fauna. *Mar Ecol Prog Ser* 79:127–131
- Seibel BA, Walsh PJ (2003) Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. *J Exp Biol* 206:641–650
- Shirayama Y, Thornton H (2005) Effects of increased atmospheric CO<sub>2</sub> on shallow water marine benthos. *J Geophys Res* 110:C09S08, doi:10.1029/2004JC002618
- Spicer JI (1995) Oxygen and acid-base status of the sea urchin *Psammechinus miliaris* during environmental hypoxia. *Mar Biol* 124:71–76
- Spicer JI, Taylor AC, Hill AD (1988) Acid-base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion. *Mar Biol* 99:527–534
- Spicer JI, Raffo A, Widdicombe S (2006) Influence of CO<sub>2</sub>-related seawater acidification on extracellular acid-base balance in the velvet swimming crab *Necora puber*. *Mar Biol* 151:1117–1125
- Takeuchi K, Fujioka Y, Kawasaki Y (1997) Impacts of high concentration of CO<sub>2</sub> on marine organisms; a modification of CO<sub>2</sub> ocean sequestration. *Energy Convers Manage* 38: S337–S341 Suppl. S
- Tjernshaugen A (2008) Political commitment to CO<sub>2</sub> capture and storage: evidence from government RD&D budgets. *Mitig Adapt Strategies Global Change* 13:1–21
- Townsend M (2006) Biodiversity and ecosystem functioning: exploring the relationship for subtidal marine benthic fauna. PhD thesis, University of York
- Ueda N, Tsutsumi H, Yamada M, Takeuchi R, Kido K (1994) Recovery of the marine bottom environment of a Japanese bay. *Mar Pollut Bull* 28:676–682
- Verardo DJ, Froelich PN, McIntyre A (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. *Deep Sea Res I* 37:157–165
- Warwick RM, Platt HM, Somerfield PJ (1998) Free-living marine nematodes, Part III. Monhysterids. Synopsis of the British Fauna (New Series) No. 53, Field Studies Council, Shrewsbury
- Wheatly MG, Henry RP (1992) Extracellular and intracellular acid-base regulation in crustaceans. *J Exp Zool* 263:127–142
- Widdicombe S, Austen MC (1998) Experimental evidence for the role of *Brissopsis lyrifera* (Forbes, 1841) as a critical species in the maintenance of benthic diversity and the modification of sediment chemistry. *J Exp Mar Biol Ecol* 228:241–255
- Widdicombe S, Austen MC (1999) Mesocosm investigation into the effects of bioturbation on the diversity and structure of a subtidal macrobenthic community. *Mar Ecol Prog Ser* 189:181–193
- Widdicombe S, Austen MC (2001) The interaction between physical disturbance and organic enrichment: an important element in structuring benthic communities. *Limnol Oceanogr* 46:1720–1733
- Widdicombe S, Needham HR (2007) Impact of CO<sub>2</sub> induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux. *Mar Ecol Prog Ser* 341:111–122
- Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *J Exp Mar Biol Ecol* 366:187–197
- Widdicombe S, Austen MC, Kendall MA, Olsgaard F, Schaanning MT, Dashfield SL, Needham HR (2004) The importance of bioturbators for diversity maintenance: the indirect effects of fishing disturbance. *Mar Ecol Prog Ser* 275:1–10
- Wood H, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proc R Soc Lond B Biol Sci* 275:1767–1773