Benthic foraminifera show some resilience to ocean acidification in the northern Gulf of California, Mexico

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ABSTRACT

Extensive CO_2 vents have been discovered in the Wagner Basin, northern Gulf of California, where they create large areas with lowered seawater pH. Such areas are suitable for investigations of long-term biological effects of ocean acidification and effects of CO_2 leakage from subsea carbon capture storage. Here, we show responses of benthic foraminifera to seawater pH gradients at 74–207 m water depth. Living (rose Bengal stained) benthic foraminifera included *Nonionella basispinata*, *Epistominella bradyana* and *Bulimina marginata*. Studies on foraminifera at CO_2 vents in the Mediterranean and off Papua New Guinea have shown dramatic long-term effects of acidified seawater. We found living calcareous benthic foraminifera in low pH conditions in the northern Gulf of California, although there was an impoverished species assemblage and evidence of post-mortem test dissolution.

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1. Introduction

Over the past 200 years, the oceans have absorbed almost a third of the carbon dioxide produced by the burning of fossil fuels (Sabine et al., 2004). This has resulted in a series of chemical changes, including lowering the pH, in a process known as ocean acidification (Caldeira and Wickett, 2003). In this context, 'acidified' refers to the process of becoming closer to chemical acidity, not that the seawater is chemically acidic (i.e. below pH 7.0). Since pre-industrial times, the ocean surface pH has fallen by approximately 0.1 units to a current day global average of 8.1, which is equivalent to a 30% increase in the concentration of hydrogen ions (Guinotte and Fabry, 2008). The current rate and magnitude of pH changes is thought to be greater than any inferred from the past 300 million years and the ocean surface pH may fall by as much as 0.77 units (Caldeira and Wickett, 2003). These chemical changes are expected to have significant consequences for marine organisms, particularly those with calcareous shells (Kroeker et al., 2010; Rodolfo-Metalpa et al., 2011). Benthic foraminifera are an important group to examine as they occur worldwide, have short life-histories, are environmentally sensitive and have an excellent fossil record (Dias et al., 2010). Much of the work conducted on foraminifera in relation to ocean acidification has involved short-term laboratory experiments (Bijma et al., 2002, 1999; Spero et al., 1997), deep sea CO_2 storage scenarios with very high levels of CO_2 (Bernhard et al., 2009a,b) or micropalaeontological investigations (Barker and Elderfield, 2002; Gonzalez-Mora et al., 2008; Moy et al., 2009; Nguyen et al., 2009).

Natural CO_2 vents can be used to study the effects of ocean acidification *in situ*, as they create areas of lower pH where carbon dioxide gas bubbles up through the sea floor (Dias et al., 2010). Some of these vents release gas (and water) at ambient seawater temperatures. The vents tend to be present on time scales of hundreds to thousands of years, so the communities present in the area are likely to be adapted to the low pH conditions, avoiding some of the problems of short-term, rapid perturbation experiments (Hall-Spencer et al., 2008). These CO_2 vents can also be used to examine the potential biological impacts of leakage from carbon capture storage (CCS) sites. There is concern that leakage from CCS will impact biological communities, as a result of perturbations in the carbonate chemistry conditions and a reduction in pH (Blackford et al., 2009). Bernhard et al. (2009a) conducted an experiment to examine the survival of deep-sea benthic foraminifera under a potential carbon dioxide sequestration scenario. They found that the survivorship of allogromiid and agglutinated foraminifera was not significantly affected, but calcareous foraminifera had a significantly lower survivorship in exposure treatments compared to controls.

Examination of benthic foraminiferal assemblages in relation to ocean acidification from natural situations is both important and lacking in the literature. Dias et al. (2010) examined living

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assemblages of benthic foraminifera around natural CO_2 vents adjacent to the island of Ischia, Italy. They found that the community changed from one dominated by calcareous forms in normal pH conditions to one dominated by agglutinated forms in lowered pH conditions. Along a gradient from normal pH (mean pH ~8.14) to acidified areas (mean pH ~7.6), species richness fell from 24 to just four species of benthic foraminifera. The reduction in species richness near to the CO_2 vents mirrored those found for larger benthic calcifying organisms (Hall-Spencer et al., 2008). In addition, Fabricius et al. (2011), who examined calcareous biota at CO_2 vents off Papua New Guinea, found that sediment at high pCO_2 sites (up to 953 ppm) was almost free of calcareous biota (including benthic foraminifera) and sites with lower pCO_2 (~444 ppm) contained many pitted or eroded tests of foraminifera. A taxonomic assessment of the assemblage was, however, not undertaken as part of the investigation at Papua New Guinea. In another study at CO_2 vents around Papua New Guinea, Uthicke and Fabricius (2010) examined the symbiont-bearing benthic foraminifera, *Marginopora vertebralis* (Quoy and Gaimard). They found *M. vertebralis* to be absent from three seep sites with pH below ~7.9, but present in densities of over 1000 m^{-2} at control sites.

The Gulf of California is a narrow marginal sea. In the northern part, upwelling creates high, but variable nutrient levels (Halfar et al., 2004). Rates of primary productivity are similar to those in the Bay of Bengal or North Africa and they are approximately 2–3 times greater than the open Atlantic at similar latitudes (Zeitzschel, 1969). The region has a high evaporation rate with strong winds and tides. Fast tidal currents that develop, particularly in spring, create a well-mixed water column which persists for most of the year (Zeitzschel, 1969). This strong mixing means that temperatures at depth in the basin are almost constant. The Wagner and Consag basins have large areas in which the pH is naturally lower due to gas seepage across the basins. In 2007 and 2010, gas vents along the Wagner Fault, in the northern Gulf of California were examined. Over 300 large, diffuse seafloor gas vents were discovered (Canet et al., 2010) causing dramatic changes to the carbonate chemistry. The vent gases are probably methane and CO_2 rich (Canet et al., 2010), although CO_2 predominates in the whole area (Prol-Ledesma et al., 2013). The Wagner and Consag basins are shallow (up to ~225 m) active basins in the north of the Gulf of California with large-scale gas venting, along the eastern edge of the basins (Canet et al., 2010).

The aims of our study were to examine; (1) the response of living benthic foraminifera and, (2) the preservation of living and dead benthic foraminifera, in different seawater pH conditions in the northern Gulf of California. Our hypothesis was that there would be an overall reduction in species richness and a reduction in calcifying species in the areas with lower pH conditions. We also expected that there would be test dissolution in the lowest pH conditions as this has been shown in other foraminiferal studies (Le Cadre et al., 2003; Moy et al., 2009).

2. Materials and methods

Samples were collected during the WAG-02 cruise aboard R/V *El Puma*. Sample stations were chosen to represent the variations in pH due to the presence of vents (Fig. 1). Sediment samples were collected using a Smith McIntyre grab. Once aboard the research vessel, 10 cm cores were taken through the sediment, which were then divided into 2 cm sub-samples. The sediment was stained with rose Bengal (1 g/L) and preserved in 4% formalin for up to 3 months, until the samples were processed.

Rosette mounted CTD casts (General Oceanics, Mark III WOOCE) were taken at every sampling station to monitor environmental parameters. Seawater samples were collected 10 m above the bottom to avoid physical damage to the CTD. To test if seawater samples collected a few centimetres above the seabed differed significantly from ones collected 10 m above the bottom, near-bottom water samples were taken at two sampling stations. A Niskin bottle was connected to an ROV arm with a hand-made system. Seawater bottom samples collected using the ROV did not differ significantly from seawater collected 10 m above the seabed using the rosette.

On-board, seawater sub-samples were immediately collected from the recovered rosette in glass bottles. The pH_T (in total scale) was measured using a meter (Methrom pH mobile) accurate to 0.01 pH units and calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (Dickson et al., 2007). Seawater samples were then passed through Whatman GF/F, treated with 0.05 ml of 50% $HgCl_2$ (Merck, Analar) and stored in the dark at 4 °C pending analysis. Three replicate 20 ml sub-samples were analysed at 25 °C using a titration system composed of a pH-meter with a Methrom pH electrode and a 1 ml automatic burette (METHROM). pH was measured at 0.02 ml increments of 0.1 N HCl. Total alkalinity (A_T) was calculated from the Gran function applied to pH from 4.2 to 3.0, as mEq l^{-1} from the slope of the curve; pH *vs.* HCl volume. Titrations of total alkalinity standards, provided by A.G. Dickson Laboratory (batch 99 and 102; Scripps Institution of Oceanography), were within 0.7 µmol kg^{-1} of the nominal value. Parameters of the carbonate system (pCO_2, CO_3^{2-}, HCO_3^-, C_T and saturation state of calcite (Ω_{Calc}) and aragonite (Ω_{Arag})) were calculated from pH_T, mean A_T, temperature and salinity using the free-access CO2SYS package (Lewis and Wallace, 1998) with the constants of (Mehrbach et al., 1973). pH is expressed on the total scale at the sampled depth of collection. A_T was virtually constant at all sites, therefore its mean was used in the calculation of the carbonate chemistry parameters (A_T = 2359.01 µmol kg^{-1}). Means of pH_T were calculated from hydrogen ion concentrations of each measurement and then re-converted back to pH (Dickson et al., 2007).

Only the core top (0–2 cm) samples were analysed as this is the segment most suitable for finding live foraminifera (Corliss, 1985). Standard micropalaeontological techniques were employed with samples washed on a 63 µm sieve (Murray, 2006). The retained fraction was left to air dry for up to 72 h. Once dry, the sediment was transferred to labelled plastic weighing vials. Prior to analysis, sediment samples were split using an Otto sediment splitter. Split samples were placed onto a brass picking tray and analysed under the stereo-binocular microscope. Individual foraminifera were dry picked using a fine paint brush and placed onto micropalaeontological slides. The foraminifera were identified, in the first instance, using (Loeblich and Tappan, 1987, 1964) and identified to species level, where possible, using Bandy (1961, 1953), Brenner (1962), McGann (2002) and Lesen (2005). The smallest split fraction was examined first. If at least 300 individuals were found within the fraction, no additional fractions were examined from this sample as 300 individuals are believed to be statistically representative of the whole sample (Pielou, 1966). If fewer than 300 individuals were found then an additional fraction was examined. This continued until at least 300 individuals had been found, or the whole sample had been examined, whichever came first. The rose Bengal stain was used to determine living individuals. Individuals were determined to have been live at the time of collection if they were stained dark magenta in at least half of their chambers. This excluded counting individuals with just a faint red dot in one chamber. As rose Bengal is a non-vital stain, it also has the potential to stain remaining protoplasm or bacteria, thereby overestimating the number of living individuals (Bernhard et al., 2006). It can also be difficult to see the staining in opaque specimens including many species of agglutinated foraminifera (Bernhard, 2000). If used carefully, however, rose Bengal can be reasonably accurate in certain
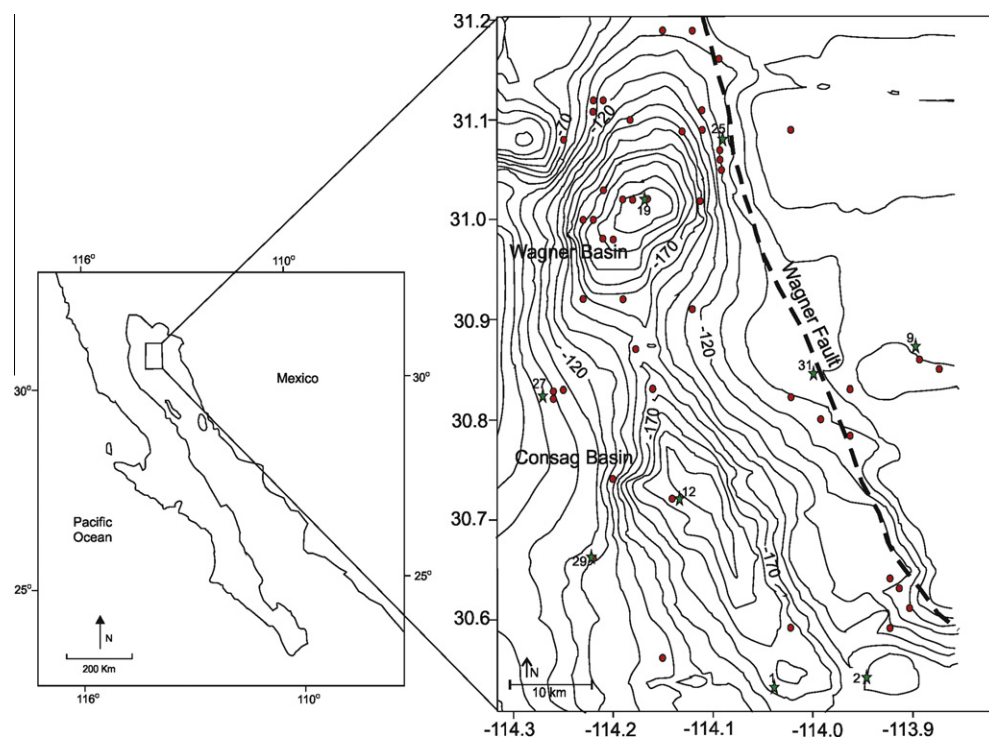
Fig. 1. Map of the Gulf of California showing the study area. The locations from which benthic foraminifera were examined are marked with stars. The number next to the star refers to the station number. Circles represent the position of CO_2 vents in the area.

Table 1
The range in carbonate chemistry parameters from stations where samples were collected in the northern Gulf of California. Measurements are single point measurements, with no replicates. Measurements were taken in July and August 2010.

Station	Depth (m)	Temp (°C)	Salinity (‰)	pH_T	Ω_{Calc}	pCO_2 (µatm)	A_T (µmol kg^{-1})	Living calcareous foraminifera	Dead calcareous foraminifera
31	80	20.6	35.4	7.88	3.60	674	2330.55	X	✔
27	88	20.4	35.4	7.86	3.46	675	–	X	✔
25	80	23.3	35.5	7.83	3.61	730	2386.43	✔	✔
9	74	22.4	35.5	7.83	3.50	735	2363.00	✔	✔
29	105	20.0	35.4	7.75	2.69	917	2382.21	✔	✔
12	195	14.0	35.2	7.69	1.90	1021	–	✔	✔
1	160	15.1	35.2	7.66	1.84	1130	2330.55	X	✔
2	160	17.3	35.4	7.63	1.89	1222	2383.26	✔	✔
19	207	15.2	35.2	7.55	1.47	1466	2330.55	✔	✔

environments (Figueira et al., 2012; Murray and Bowser, 2000) and it is inexpensive and relatively easy to use on a research cruise, where there may be limited facilities.

To view their preservation, some foraminifera were imaged using a JEOL JSM 6610 LV scanning electron microscope (SEM) with a digital imaging system. Individuals were mounted on aluminium SEM stubs and sputter coated in an Emitech K550 gold sputter coater.

The abundances of benthic foraminifera were used to calculate the Shannon–Wiener diversity index, the Fisher Alpha index and Pielou's evenness index. The Fisher Alpha index was used in addition to Shannon diversity as this is the most commonly used index in foraminiferal studies. Correlations between species richness, the number of individuals, Shannon–Wiener diversity index and environmental parameters were tested using Spearman's rank order correlation. As a means of testing whether there was a difference between stations with higher and lower pH, a Mann–Whitney rank sum test was conducted on the data. The stations were separated into two groups: those with a low pH (7.55, 7.63, 7.66, 7.69 and 7.75 ($n = 5$)) and those with a high pH (7.83, 7.83, 7.86 and 7.88 ($n = 4$)). SigmaPlot 12.0 was used to perform these analyses. Further statistical analysis was not undertaken due to limited sample numbers and sample size.

3. Results

3.1. Environmental parameters

The water chemistry data are based on single point measurements. Salinity ranged from 35.2‰ to 35.5‰ and total alkalinity ranged from 2331 to 2386 µmol kg^{-1}. The pH_T of the bottom waters ranged from 7.55 to 7.88, indicative of prevailing low pH conditions due to the presence of the CO_2 vents. The seawater at all stations was oversaturated with respect to calcite (Ω_{Calc}), the lowest Ω_{Calc} value being 1.47 (Table 1). The aragonite saturation state (Ω_{Arag}) ranged from 0.95 to 2.37, with undersaturation only at the station with the lowest pH.

3.2. Species composition

3.2.1. Living assemblages

There were low numbers of living individuals present in all samples and three of the samples had no living individuals (Table 2). Two of the samples with no living individuals were stations with the highest pH (7.86 and 7.88 units). Of the living

Table 2
A list of the species of benthic foraminifera found within the living (stained) assemblage. Numbers per gram of sediment are reported along with the percentage contribution. Numbers per gram were calculated from the dry sediment weight of the > 63 μm fraction.

Species	pH 7.55		7.63		7.66		7.69		7.75		7.83		7.83		7.86		7.88	
	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%	Live per (g)	%
Ammonia beccarii	–	–	–	–	–	–	–	–	12	38	–	–	–	–	–	–	–	–
Bolivina (inflated sutures)	–	–	50	18	–	–	–	–	2	6	–	–	2	17	–	–	–	–
Bolivina acutula	–	–	25	9	–	–	–	–	–	–	5	100	–	–	–	–	–	–
Bolivina sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bulimina denudata	–	–	50	18	–	–	–	–	–	–	–	–	2	17	–	–	–	–
Bulimina marginata	–	–	–	–	–	–	–	–	4	13	–	–	–	–	–	–	–	–
Elphidium excavatum	–	–	–	–	–	–	1	50	4	13	–	–	–	–	–	–	–	–
Epistomella bradyana	–	–	125	45	–	–	–	–	4	13	–	–	–	–	–	–	–	–
Hanzawaia nitidula	–	–	–	–	–	–	1	50	2	6	–	–	2	17	–	–	–	–
Nonionella basispinata	155	100	25	9	–	–	–	–	–	–	–	–	2	17	–	–	–	–
Reophax sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	2	17	–	–	–	–
Indeterminate calcareous sp.11	–	–	–	–	–	100	–	–	–	–	–	–	–	–	–	–	–	–
Indeterminate agglutinate sp. 10	–	–	–	–	–	–	–	–	2	6	–	–	–	–	–	–	–	–
Indeterminate agglutinate sp. 7	–	–	–	–	–	–	–	–	–	–	–	–	2	17	–	–	–	–
Indeterminate agglutinate sp. 8	–	–	–	–	–	–	–	–	2	6	–	–	–	–	–	–	–	–

individuals found, the most abundant species were *Nonionella basispinata* (Cushman and Moyer) (37.7%), *Epistominella bradyana* (Cushman) (27.0%) and *Bulimina marginata* (d'Orbigny) (11.3%). The numbers in brackets refer to the contribution of each species to the total number of living individuals.

Although some calcareous benthic foraminifera were present within the samples, the living assemblage was impoverished. The percentage of living individuals out of the total species assemblage (living and dead) varied from 0% to 20%, with the highest percentage of living individuals occurring in the lowest pH sample. In addition to calcareous foraminifera, four species of agglutinated foraminifera with mineralised or proteinaceous cement were found within the living assemblage. These were found from two different sample stations. Dry picking may have resulted in the loss of some of the more fragile agglutinated taxa (Murray and Bowser, 2000), but these taxa were not the focus of this investigation. Dias et al. (2010) who also used the method of dry picking, found that the proportion of agglutinated taxa increased as pH decreased.

The highest species richness for any one sample was eight species. In those samples that did have living individuals, the Shannon–Wiener diversity index ranged from 0.00 to 1.84, suggesting a low diversity in all samples. The number of individuals per gram appeared to increase with an increase in pH, calcite saturation state and water temperature and decrease as water depth increased (Fig. 2). These apparent trends, however, were not statistically significant, although the low number of living specimens made it difficult to assess fully. Spearman's rank order correlation revealed that there was no significant correlation between species richness, the number of individuals per gram or the Shannon–Weiner diversity index and pH_T, Ω_{Cal}, water depth or temperature ($p > 0.050$). The results of the Mann–Whitney rank sum test revealed that there was no significant difference in species richness ($p = 0.413$) or the number of individuals per gram ($p = 0.286$) between the low and high pH groups.

3.2.2. Dead assemblages

Dead benthic foraminifera present in the top 2 cm of the sediment contained a selection of mainly calcareous taxa (including *E. bradyana* (54.5%), *B. marginata* (13.5%), *Eponides* sp. (7.4%), *Elphidium excavatum* (Terquem) (6.4%) and *N. basispinata* (3.8%)) (Table 3). The numbers in brackets refer to the contribution of each species to the total number of dead individuals. These species are considered to constitute a normal composition for these water depths with the exception of *E. excavatum* which is typically a shallow water species occurring in water depths between 0 and 50 m (Murray, 1991). The presence of *E. excavatum* suggests possible down-slope transportation in the area.

Agglutinated taxa with mineralised or proteinaceous cement were found in seven of the samples and those with calcareous cement were found in only two samples. The only sample with no agglutinated foraminifera was the station with the lowest pH. As mentioned above, dry picking may have resulted in the loss of some of the more fragile agglutinated taxa (Murray and Bowser, 2000).

The highest species richness for any one sample was 24 species and the lowest was nine species. Species richness appeared to increase with an increase in pH, calcite saturation state and water temperature and decrease as water depth increased (Fig. 3). These patterns were statistically significant and Spearman's rank order correlation revealed that species richness increased as pH ($r = 0.79$, $p = 0.009$, $n = 9$), Ω_{Calc} ($r = 0.84$, $p = 0.002$, $n = 9$) and water temperature ($r = 0.88$, $p \leqslant 0.001$, $n = 9$) increased and decreased as water depth increased ($r = -0.96$, $p \leqslant 0.001$, $n = 9$). There was no significant correlation between the number of individuals per gram, the Shannon–Weiner diversity index or the Fisher Alpha index and pH_T, Ω_{Calc}, water depth or temperature ($p > 0.050$). These

results suggest that there was a reduction in species richness in the dead assemblage as pH decreased, but no statistically significant change in the number of individuals.

The results of the Mann–Whitney rank sum test revealed there to be a significant difference in species richness ($p = 0.016$) between the low and high pH groups. There was no statistically significant difference in the number of individuals per gram of sediment ($p = 0.730$), the Shannon–Wiener diversity index ($p = 0.556$), the Fisher Alpha index ($p = 0.730$), the number of calcareous species per gram ($p = 0.730$) or the number of agglutinated foraminifera per gram ($p = 0.286$) between the two groups (low and high pH).

3.3. Scanning electron microscopy

A total of 40 different individuals (live and dead) were examined under the SEM, belonging to six species. The wall detail was examined under a higher magnification (up to x8000) in 26 of these individuals. The wall detail of the final chamber was examined if possible. Some individuals had their last chamber broken off, in which case the penultimate chamber was examined. Some dissolution was determined to have occurred if the foraminifera test showed etching, pitting, fragmentation or enlarged pores.

Unfortunately, the low number of living individuals limited the number that could be viewed under the SEM. Out of the 40 individuals examined under the SEM, 10 were living. Some of the living foraminifera (30%) showed signs of dissolution, suggesting that they were able to live in the low pH conditions, but their tests were beginning to dissolve. Alternatively, the dissolution could be early post-mortem. Most of the dead individuals (60%) showed signs of dissolution and many of these showed severe signs of dissolution, with extensive pitting and fragmentation.

Although there were signs of dissolution in some of the foraminifera, there were no deformities (such as abnormally shaped chambers) in any of the individuals examined under the SEM

(Fig. 4). This was also true in Ischia samples studied by Dias et al. (2010). Circular borings with a distinct profile were found in a few of the individuals examined (Fig. 5), which are thought to be caused by gastropods belonging to the families Naticidae and Muricidae (Maddocks, 1988). These are not, however, a dissolution feature and indicate that the specimen was almost certainly living when it was bored.

4. Discussion

Calcifying benthic foraminifera were present in pH conditions ranging from 7.55 to 7.88 near CO_2 vents in the northern Gulf of California. Calcareous foraminifera dominated the species composition at the lowest pH stations. Our hypothesis that there would be a reduction in species richness and the number of calcifying species for the living assemblage in the lower pH conditions was not supported. Although the species richness and number of individuals was low, this was the case for all stations. The hypothesis that there would be evidence of test dissolution in calcareous benthic foraminifera was supported.

The range in pH between the stations was 0.33 units. Although this is less than the total decrease in pH (0.77 units) that is predicted to occur due to the anthropogenic burning of fossil fuels (Caldeira and Wickett, 2003), it still represents a large range in the concentration of hydrogen ions. The range in pH (0.33 units) is greater than the mean pH reductions (<0.05 units) measured in bottom waters during an experiment assessing the impact of injected carbon dioxide on deep-sea benthic foraminifera (Bernhard et al., 2009a) and is similar to those expected along a dilution gradient, under carbon sequestration options (Bernhard et al., 2009a). The highest pH recorded was 7.88, this below the value of 8.1, which is considered to be a global average for surface waters (Guinotte and Fabry, 2008; Raven et al., 2005), but it is expected that infaunal benthic foraminifera will experience lower pH conditions within the sediment pore water. At the locations from which
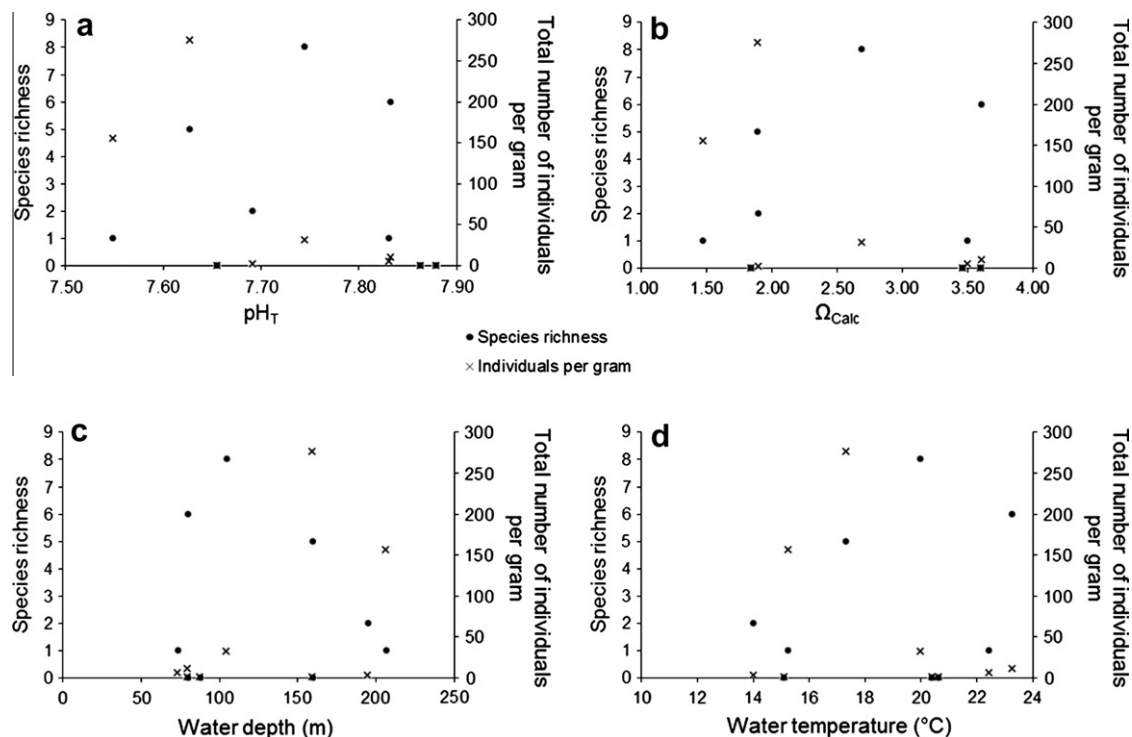

Fig. 2. The species richness and the number of individuals per gram for the living (stained) assemblage plotted against: (a) pH_T, (b) calcite saturation state, (c) water depth and (d) water temperature.

Table 3
A list of the species of benthic foraminifera found within the dead (not stained) assemblage. Numbers per gram of sediment are reported along with the percentage contribution. Numbers per gram were calculated from the dry sediment weight of the >63 μm fraction.

Species	pH																	
	7.55		7.63		7.66		7.69		7.75		7.83		7.83		7.86		7.88	
	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%	Dead per g	%
Ammonia beccarii	–	–	–	–	16	15.8	1	0.9	242	49.0	5	0.3	0	0.0	64	1.8	6	5.7
Bolivina (inflated sutures)	–	–	150	2.5	2	1.8	–	–	–	–	–	–	–	–	–	–	1	0.5
Bolivina acuminata/spinosa	–	–	100	1.7	11	11.4	5	4.5	2	0.4	40	2.7	2	0.8	43	1.2	1	0.5
Bolivina acutula	–	–	100	1.7	–	–	3	2.7	–	–	111	7.4	18	8.3	64	1.8	2	1.5
Bolivina sp. 1	–	–	25	0.4	2	1.8	–	–	–	–	20	1.4	–	–	32	0.9	–	–
Bolivina sp. 2	–	–	–	–	–	–	–	–	–	–	10	0.7	–	–	–	–	–	–
Bolivina sp. 3	–	–	–	–	–	–	–	–	–	–	25	1.7	–	–	–	–	–	–
Bolivina sp. 4	70	11.6	–	–	–	–	1	0.9	2	0.4	5	0.3	–	–	32	0.9	–	–
Bolivina sp. 5	–	–	–	–	–	–	–	–	4	0.8	–	–	–	–	–	–	–	–
Buccella tenerrima	–	–	–	–	–	–	–	–	–	–	5	0.3	–	–	74	2.2	–	–
Bulimina denudata	–	–	–	–	–	–	–	–	2	0.4	–	–	–	–	21	0.6	–	–
Bulimina marginata	–	–	925	15.4	10	9.6	3	2.7	36	7.4	106	7.1	97	44.4	521	15.1	10	9.3
Bulimina sp. 1	–	–	–	–	1	0.9	–	–	–	–	5	0.3	–	–	–	–	–	–
Bulimina sp. 2	–	–	25	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cancris auricula	28	4.7	–	–	–	–	1	0.9	8	1.6	20	1.4	8	3.8	21	0.6	1	0.5
Cassidulina sp. 1	28	4.7	–	–	–	–	1	0.9	2	0.4	–	–	–	–	11	0.3	–	–
Cassidulina sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	43	1.2	–	–
Elphidium excavatum	14	2.3	100	1.7	33	33.3	9	8.1	115	23.3	20	1.4	20	9.0	500	14.5	3	3.1
Epistomella bradyana	296	48.8	3750	62.5	16	15.8	72	67.6	54	10.9	975	65.2	39	18.0	1511	43.7	65	60.3
Eplidium sp. 1	–	–	–	–	–	–	1	0.9	–	–	–	–	–	–	–	–	–	–
Eponides sp. 1	42	7.0	700	11.7	6	6.1	8	7.2	13	2.7	121	8.1	11	5.3	21	0.6	17	16.0
Eponides sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	2	0.8	–	–	–	–
Hanzawaia nitidula	14	2.3	–	–	–	–	2	1.8	6	1.2	5	0.3	–	–	106	3.1	–	–
Lagena sp. 1	–	–	–	–	–	–	–	–	–	–	5	0.3	2	0.8	–	–	–	–
Lagena sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0.5
Lagena sp. 3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0.5
Lenticulina	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	0.3	–	–
Loxostomum pseudoberyrichi	–	–	–	–	–	–	–	–	–	–	–	–	2	0.8	–	–	–	–
Nonionella basispinata	99	16.3	25	0.4	2	1.8	–	–	6	1.2	5	0.3	10	4.5	330	9.5	–	–
Nonionella sp. 1	14	2.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Reophax sp. 1	–	–	–	–	–	–	–	–	–	–	5	0.3	3	1.5	–	–	1	0.5
Reophax sp. 2	–	–	–	–	–	–	–	–	–	–	–	–	3	1.5	–	–	–	–
Texturlaria sp. 1	–	–	25	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Texturlaria sp. 2	–	–	25	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Texturlaria sp. 3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	0.3	–	–
Trochammina sp. 1	–	–	25	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trochammina sp. 2	–	–	25	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trochammina sp. 3	–	–	–	–	–	–	1	0.9	–	–	–	–	–	–	–	–	–	–
Trochammina sp. 4	–	–	–	–	–	–	–	–	2	0.4	–	–	–	–	–	–	–	–
Trochammina sp. 5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0.5
Uvigerina excellens	–	–	–	–	2	1.8	–	–	–	–	5	0.3	–	–	43	1.2	1	0.5
Veleroninoides sp.	–	–	–	–	–	–	–	–	–	–	–	–	2	0.8	–	–	–	–
Indeterminate calcareous sp. 1	–	–	–	–	1	0.9	–	–	–	–	–	–	–	–	–	–	–	–
Indeterminate calcareous sp. 2	–	–	–	–	1	0.9	–	–	–	–	–	–	–	–	–	–	–	–
Indeterminate calcareous sp. 3	–	–	–	–	–	–	–	–	–	–	5	0.3	–	–	–	–	–	–
Indeterminate calcareous sp. 4	–	–	–	–	–	–	–	–	–	–	5	0.3	–	–	–	–	–	–
Indeterminate calcareous sp. 6	–	–	–	–	–	–	–	–	–	–	5	0.3	–	–	–	–	–	–
Indeterminate calcareous sp. 7	–	–	–	–	–	–	–	–	–	–	–	–	10	4.5	–	–	–	–
Indeterminate calcareous sp. 8	–	–	–	–	–	–	–	–	–	–	–	–	3	1.5	–	–	–	–
Indeterminate calcareous sp. 9	–	–	–	–	–	–	–	–	–	–	–	–	2	0.8	–	–	–	–
Indeterminate calcareous sp. 10	–	–	–	–	–	–	–	–	–	–	–	–	2	0.8	–	–	–	–

benthic foraminifera were examined, pore water pH ranged from 6.06 to 7.34 (Ruth Esther Villanueva-Estrada, unpublished data).

Some shallow water species *Ammonia beccarii* (Linné) and *E. excavatum* were found within the species assemblages. *A. beccarii* and *E. excavatum* typically live in water depths between 0 and 50 m (Murray, 1991). The presence these species suggests possible down-slope transportation in the area. Only a very small percentage of *A. beccarii* (3.3%) and *E. excavatum* (0.6%) were living. This suggests that the majority of the individuals had been washed into the sample sites and were not living in the vicinity of the vents. The one living *A. beccarii* examined under the SEM showed some signs of dissolution. Of the two living *E. excavatum* examined under the SEM, neither showed signs of dissolution.

Living calcareous species also included a mixture of infaunal and epifaunal taxa expected from these water depths such as *N. basispinata* (infaunal), *B. marginata* (infaunal) and *E. bradyana* (epifaunal) (Murray, 1991). It is perhaps surprising that more agglutinated foraminifera were not found within the species assemblage. Bandy (1961), who examined foraminifera from different depths and locations in the Gulf of California also reported low percentage contributions of agglutinated foraminifera. In the lowest pH conditions examined around CO_2 vents at Ischia, Italy, Dias et al. (2010) found that agglutinated foraminifera dominated the assemblage, although there was a low species diversity.

There were no living *Eponides* species within the samples, whereas these dominated the dead assemblage. This suggests that these species were unable to survive in the low pH conditions or that they were not actually autochthonous and had been transported in from elsewhere. *Eponides* have been reported as dominating species assemblages in low oxygen environments (0.25–1.1 ml l^{-1} O_2) (Douglas and Heitman, 1979). Pore waters in anoxic sediments can sometimes be alkaline as a result of the sulphate-reducing bacteria (Murray, 1991), which involves the reduction of CO_2 and the formation of hydrocarbons (Wright and Colling, 1995). This may explain why living *Eponides* species, which are typically tolerant of low oxygen, alkaline conditions, were not found in low pH conditions. Other taxa found within the living assemblage, however, such as *Bolivina* and *Bulimina* have also been found in low oxygen environments (Douglas and Heitman, 1979).

Dias et al. (2010) found a dramatic reduction in benthic foraminiferal diversity and abundance near to CO_2 vents around Ischia Island, Italy. In contrast to our findings, no living calcareous foraminifera were found below pH $\sim$ 7.6 at Ischia (Dias et al., 2010) and Uthicke and Fabricius (2012) found no *M. vertebralis* at Papua New Guinea CO_2 seep sites with pH below $\sim$7.9. Fabricius et al. (2011) found that high pCO$_2$ had a negative impact on the number of calcareous benthic foraminifera at Papua New Guinea. Although we found some living calcareous benthic foraminifera, they were present in low numbers. Fabricius et al. (2011), however, did not distinguish between living and dead individuals. If the total assemblage (living and dead individuals) is considered from the northern Gulf of California samples, then disparity with Fabricius et al. (2011) seems to be even greater, as there was no reduction in the number of calcareous benthic foraminifera in the lowest pH sites.

Some living benthic foraminifera may be able to survive the low pH conditions in the northern Gulf of California due to the supply of nutrients and the availability of food. The northern Gulf of California is characterised by abundant nutrients which are carried to the surface through upwelling and tidal mixing (Halfar et al., 2004, 2006; Zeitzschel, 1969). A plentiful supply of food for foraminifera in the northern Gulf of California may enable them to persist in the stress of a low pH environment. *Bolivina* and *Nonionella*, two taxa found in this investigation, are typically found in organic rich environments (Kennett et al., 2000; Rathburn et al., 2000). In the high nutrient conditions of Kiel Fjord, calcifying invertebrates were found to dominate the macrobenthic

Indeterminate calcareous sp. 12				
Indeterminate calcareous sp. 13	0.5	1	0.3	
Indeterminate calcareous sp. 14	0.5	1		
Indeterminate agglutinate sp. 1			0.3	0.9
Indeterminate agglutinate sp. 2	11	0.8	0.3	5
Indeterminate agglutinate sp. 3		0.8	2	5
Indeterminate agglutinate sp. 6			2	1.2
Indeterminate agglutinate sp. 8				6
Indeterminate agglutinate sp. 9			0.4	2
Indeterminate agglutinate sp. 11	1.5	2		

community despite low pH conditions (Thomsen et al., 2010). Conversely, the Mediterranean Sea is an oligotrophic area in which nutrients decrease towards the east. Low pH conditions at Ischia will be an additional stress to that of limited food supply. Bacteria on the seagrass at Ischia, which would be expected to provide a food source for epiphytic foraminifera (Muller and Lee, 1969), are reported to have low densities (Novak, 1982; Velimirov, 1986; Velimirov et al., 1984). Limited food supply at Ischia could, therefore, be a reason for differences in the presence of living calcareous benthic foraminifera in the northern Gulf of California and Ischia.

Rodolfo-Metalpa et al. (2010) found that increased temperature and decreased pH acted synergistically to reduce calcification in the bryozoan *Myriapora truncata* at CO_2 vents around Ischia. Further work revealed that the effects of ocean acidification on molluscs and corals were also exacerbated by high temperatures (Rodolfo-Metalpa et al., 2011). If the same principle applies to calcareous benthic foraminifera, this could be a possible explanation for their presence in the northern Gulf of California, but not Ischia or Papua New Guinea where seawater temperatures are higher. The shallow waters of Ischia and Papua New Guinea can warm quickly, resulting in temperatures up to 30 °C at Ischia (Rodolfo-Metalpa et al., 2011). Temperature can control the major distribution patterns of shallow water foraminifera (Murray, 2006) and the higher seawater temperatures at Ischia may provide an additional stress to the benthic foraminifera. It may be the extent of the change in temperature, above or below the mean that is important, rather than the absolute temperature. The deeper environment and strong mixing in the northern Gulf of California means that temperature fluctuations are likely to be less severe than those in the shallow waters around Ischia and Papua New Guinea.

Variability in the calcite saturation state could also affect the ability of calcareous benthic foraminifera to survive in low pH conditions in the northern Gulf of California. The variability in Ω_{Calc} in the northern Gulf of California and at Papua New Guinea is unknown, but there is a high degree of variability at Ischia (Hall-Spencer et al., 2008; Kerrison et al., 2011). The emissions from the vents in the Wagner Basin are continuous and the pH recorded in this study roughly corresponds to pH values measured during the previous WAG-01 cruise. It is, therefore, expected that the saturation state will be fairly stable. In addition, the lack of strong temperature variations in the northern Gulf of California could mean that there is little variation in Ω_{Calc}. It may be that foraminifera cannot survive in the variable saturation states at Ischia, and possibly Papua New Guinea, but more stable saturation states in the northern Gulf of California allow some to survive.

It is thought that in many organisms calcium carbonate saturation exerts the strongest control on calcification. As the saturation state reduces, the energetic cost of creating and maintaining biogenic calcium carbonate is expected to increase (de Nooijer et al., 2009a,b; Guinotte and Fabry, 2008; Orr et al., 2005; Riebesell et al., 2000). Differences in the saturation state were proposed as a possible explanation for differences in the abundance of the coccolithophore *Emiliania huxleyi* between the Baltic Sea and the Black Sea (Tyrrell et al., 2008). *E. huxleyi* were found to be abundant in the Black Sea, but absent from the Baltic Sea, which is surprising given their abundance in nearby waters at similar latitudes. Although differences in environmental factors, such as salinity or silicate concentration, could be an explanation for their absence, undersaturation with respect to aragonite and calcite in the Baltic Sea, but not the Black Sea, could also be an explanation. It is possible, however, that calcification rates in foraminifera are not related to calcite saturation state (de Villiers, 2004). Highest foraminifera shell weights were found not to have a straightforward relationship with saturation state; instead calcification appeared to be a function of the complex interplay of environmental parameters (de Villiers, 2004). This argues against the role that saturation state can play in determining the resilience of benthic foraminifera to ocean acidification.
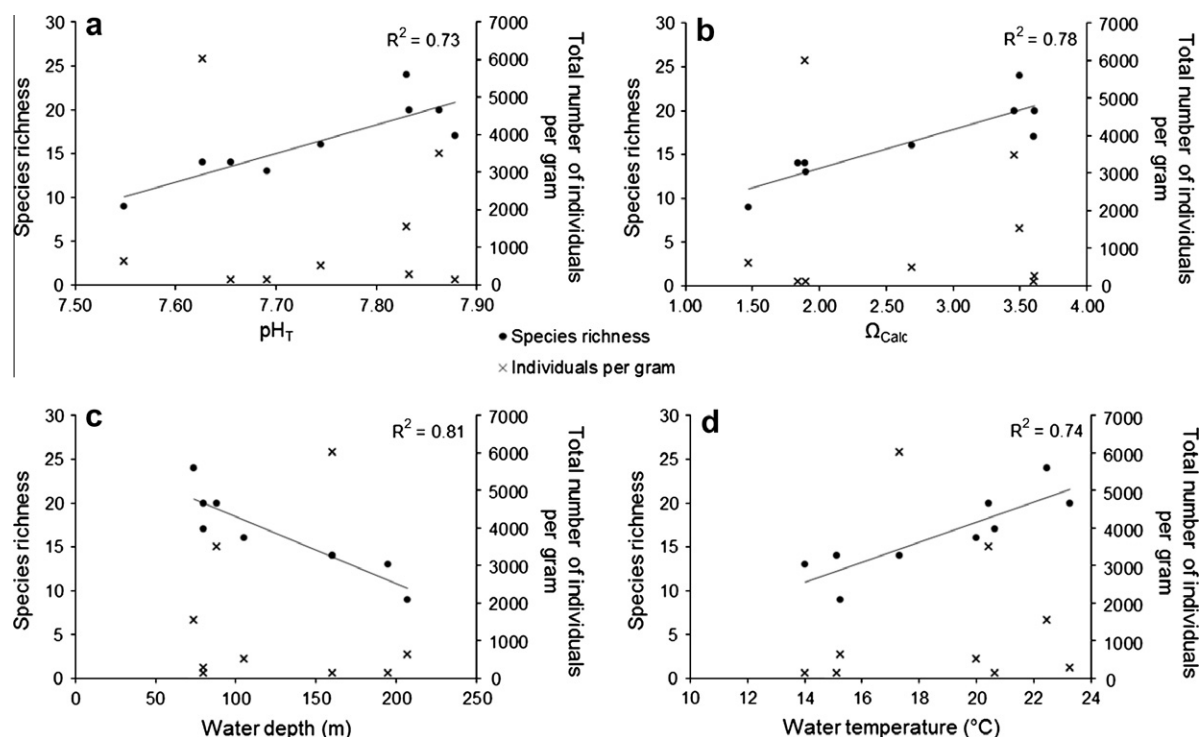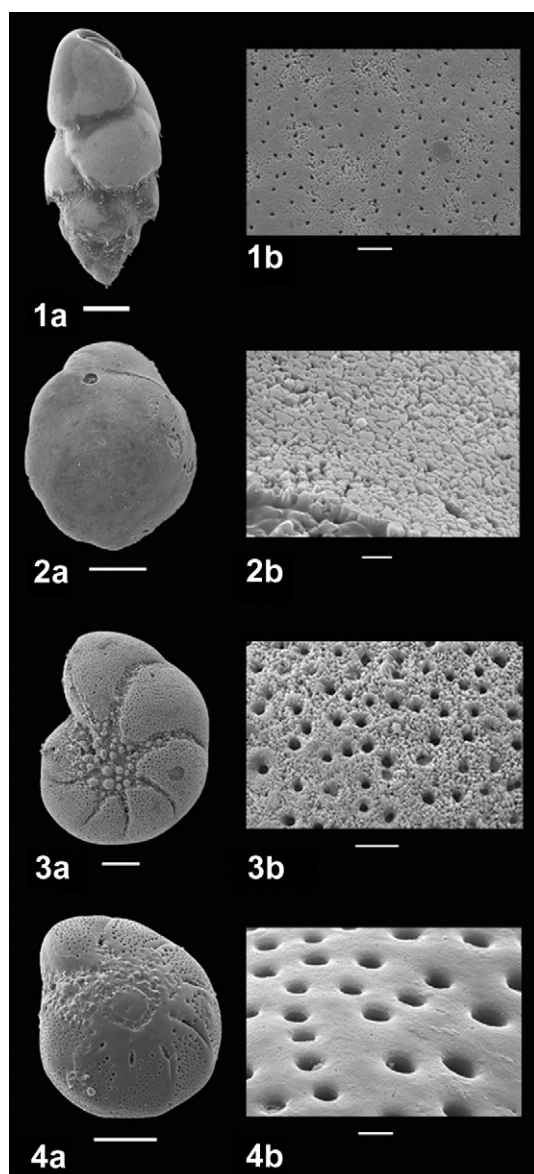

Fig. 3. The species richness and the number of individuals per gram for the dead assemblage plotted against: (a) pH_T, (b) calcite saturation state, (c) water depth and (d) water temperature. A linear trend line has been plotted on the graph for the species richness data points along with the R^2 value.

during their intracellular passage which elevated CO_3^{2-} concentration. The energy expenditure of the foraminifera, needed to reach the required pH and maintain the same calcification rate, would still be determined by the initial saturation state of the seawater in the vacuole. The need for increased energy expenditure, in order to maintain the same rate of calcification, may explain why some of the living foraminifera examined under the SEM showed signs of dissolution. Although the foraminifera were still living in the low pH sites, they may not have had the required energy to maintain their calcification rate.

It is also possible that the calcareous benthic foraminifera experience increased dissolution of their tests. Rodolfo-Metalpa et al. (2011) found that when corals and molluscs were transplanted near to CO_2 vents in the Mediterranean, they were still able to calcify and grow faster, but dissolution rates of their calcium carbonate skeletons increased. This may also be the case for benthic foraminifera in the northern Gulf of California. It may be that in some low pH environments, although the foraminifera are still able to produce their calcium carbonate tests, they have thinner or smaller tests and reduced shell weights. This was found in a study of planktic foraminifera in the Southern Ocean (Moy et al., 2009). When comparing sediment trap data with sediments of pre-industrial age, Moy et al. (2009) found approximately a 30–35% reduction in the shell weight of *Globigerina bulloides* (d'Orbigny).

Our results indicate that some benthic foraminifera might be able to survive in low pH conditions due to carbon dioxide injection at a CCS site. It is likely, however, that the species assemblage will be reduced and the living foraminifera may experience test dissolution. This is similar to the findings of Bernhard et al. (2009a) who found that calcareous foraminifera had significantly lower survivorship under a potential carbon dioxide sequestration scenario. The examination of foraminifera from an area naturally lower in pH means that any foraminifera living in the area are likely to have experienced the low pH conditions throughout their life span.

Foraminifera must have survived in high CO_2 environments that occurred during the Cretaceous–Paleogene "greenhouse" world

Fig. 4. Scanning electron microscope images of some living (stained) benthic foraminifera with wall details shown. (1a) *Bulimina marginata* from station 29 (pH 7.75), (b) wall detail; (2a) *Epistominella bradyana* from station 29 (pH 7.75), (b) wall detail; (3a) *Elphidium excavatum* from station 29 (pH 7.75), (b) wall detail; (4a) *E. excavatum* from station 12 (pH 7.69), (b) wall detail. The scale bars for (1a, 2a, 3a and 4a) are 50 µm, and the scale bars for (1b, 2b and 4b) are 2 µm; the scale bar for (3b) is 5 µm.

Foraminifera are thought to promote calcification by elevating their intracellular pH (de Nooijer et al., 2009a,b, 2008). A reduction in pH would, therefore, be expected to increase the amount of energy required to elevate intracellular pH to levels required for calcification. Alternatively, if the organisms are unable to elevate intracellular pH to the required levels, it is expected that there will be reduced carbonate concentrations for precipitation (de Nooijer et al., 2009b). Either of these two scenarios is likely to lead to a reduction in calcification. This view might be too simplistic and the methods used by de Nooijer et al. (2009b, 2008) are likely to lead to reduced resolution and unreliable pH measurements (Bentov et al., 2009). Bentov et al. (2009) observed that Ca^{2+} was supplied to the site of calcification in *Amphistegina lobifera* (Larsen), the benthic foraminifera, through the transport of seawater in vacuoles rather than through membrane ion transporters. The seawater vacuoles underwent alkalisation (to a pH of 8.7)

Fig. 5. An SEM image of *Epistominella bradyana* from station 9 (pH 7.83) with evidence of boring features. The roundness and evenness of the features suggest a biological origin, thought to be caused by gastropods belonging to the families Naticidae and Muricidae. This specimen was not living. The scale bar is 50 µm.

where atmospheric pCO$_2$ was very much higher (estimates have ranged from ∼1000 ppm up to ∼4000 ppm), but with calcareous foraminifera apparently thriving (Andrews et al., 1995; Berner, 1990; Berner et al., 1983; Bice and Norris, 2002; Caldeira and Rampino, 1991; Kintisch, 2006; Royer et al., 2004; Tyrrell and Zeebe, 2004; Walker et al., 1981). Concentrations of CO$_3^{2-}$ are thought to have almost quadrupled since the Cretaceous and pH has increased, whilst the calcium carbonate saturation state has varied very little (Tyrrell and Zeebe, 2004). This suggests that foraminifera were able to survive in the low pH conditions (∼0.6 to 0.7 pH units lower than present day) of the Cretaceous because the calcite saturation state remained high (Stanley and Hardie, 1998). It is possible that the saturation state was decoupled from pH and remained high (Hönisch et al., 2012; Ridgwell and Schmidt, 2010). In events involving a geologically rapid release of CO$_2$, such as is occurring in the present day, the balances between sources (weathering) and sinks (CaCO$_3$ burial) of calcium will not be able to respond fast enough to regulate Ω. This will result in a coupled decline of both pH and Ω (Ridgwell and Schmidt, 2010).

5. Conclusions

Examination of calcareous benthic foraminifera collected from around natural CO$_2$ vents in the northern Gulf of California has revealed that they show some resilience to low pH conditions. Living calcareous benthic foraminifera were present in pH conditions as low as 7.55 units, albeit in low numbers. Although these results have some similarities to the findings from other shallow water CO$_2$ vents at Papua New Guinea (Fabricius et al., 2011) and Ischia (Dias et al., 2010), calcareous species were not found in the lowest pH conditions (∼7.6 units) examined at Ischia. Differences in nutrient concentrations and saturation state variability in the northern Gulf of California may be a possible explanation for the discrepancies. There was some evidence of dissolution in individuals examined under the SEM at relatively high magnification. This could be a result of the foraminifera not being able to meet the increased energy requirements to maintain the same calcification rate under low pH conditions, or a result of increased dissolution of their tests.

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