

Decline in Coccolithophore Diversity and Impact on Coccolith Morphogenesis Along a Natural CO₂ Gradient

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Abstract. A natural pH gradient caused by marine CO₂ seeps off Vulcano Island (Italy) was used to assess the effects of ocean acidification on coccolithophores, which are abundant planktonic unicellular calcifiers. Such seeps are used as natural laboratories to study the effects of ocean acidification on marine ecosystems, since they cause long-term changes in seawater carbonate chemistry and pH, exposing the organisms to elevated CO₂ concentrations and therefore mimicking future scenarios. Previous work at CO₂ seeps has focused exclusively on benthic organisms. Here we show progressive depletion of 27 coccolithophore species, in terms of cell concentrations and diversity, along a calcite saturation gradient from Ω_{calcite} 6.4 to <1. Water collected close to the main CO₂ seeps had the highest concentrations of malformed *Emiliana huxleyi*. These observations add to a growing body of evidence that ocean acidification may benefit some algae but will likely cause marine biodiversity loss, especially by impacting calcifying species, which are affected as carbonate saturation falls.

Introduction

Human-induced CO₂ emissions are causing rapid changes in surface ocean carbonate chemistry. These

changes correspond to increases of both [CO₂] and [H⁺], and decreases of pH, [CO₃²⁻], and of carbonate saturation states (Ω). This process, called ocean acidification, has raised concerns about long-term effects on marine ecosystems (Caldeira and Wickett, 2003; Kroecker *et al.*, 2013a). High CO₂ concentrations affect the metabolism, growth, calcification, and behavior of many marine organisms (Rodolfo-Metalpa *et al.*, 2011; Kroecker *et al.*, 2013a). Some photosynthetic organisms benefit from increased levels of CO₂, although many calcified species are adversely affected by corrosion or competition from non-calcified forms (Porzio *et al.*, 2011; Johnson *et al.*, 2013; Kroecker *et al.*, 2013b).

Coccolithophores are a major group of unicellular marine calcifying algae secreting calcite plates (coccoliths) covering the cell (coccosphere). Coccolithophores have been present since the Mesozoic (Bown, 1998). These phytoplankton are at the base of the food web and have been a focus of numerous ocean acidification studies because they are a major carbonate producer (Beare *et al.*, 2013). They are an important component of the marine carbon cycle, contributing to carbonate and carbon export, acting as “ballast” by increasing particle density and sinking speeds, and providing protection from remineralization (Ziveri *et al.*, 2007; Wilson *et al.*, 2012). The vast majority of ocean acidification studies on plankton have been performed in culture and mesocosm experiments, in which the carbonate system has been manipulated by the addition of acid (*e.g.*, HCl) or by bubbling of CO₂ (Riebesell *et al.*, 2008). Results

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of these short-term studies show a range of coccolithophore calcification (particulate inorganic carbon) and productivity (particulate organic carbon) responses to changes in seawater carbonate chemistry (Riebesell *et al.*, 2000; Zondervan, 2007; Iglesias-Rodriguez *et al.*, 2008; Langer *et al.*, 2009). It is not known whether these organisms will evolve or adapt to long-term acidification of the oceans (Beare *et al.*, 2013).

An interest in the long-term effects of elevated $p\text{CO}_2$ has prompted *in situ* studies. Recent studies in the Mediterranean demonstrated that coccolithophore distribution and mass are strongly related to the carbonate chemistry of the seawater (Meier *et al.*, 2014; Oviedo *et al.*, 2014). Tyrrell *et al.* (2008) reported that coccolithophores are common in the Black Sea, where surface waters are saturated with carbonate year-round, but absent from the Baltic Sea, which has seasonal carbonate undersaturation. However, because salinity, temperature, and light vary with carbonate saturation in these two seas it is difficult to disentangle the effects of these potentially confounding factors. Other studies have highlighted the complexity of decoupling multiple environmental changes on coccolithophore calcification (*e.g.*, Smith *et al.*, 2009; Horigome *et al.*, 2014).

A comparison of water samples and sediments across a variety of locations has shown that the weight of individual coccoliths (mainly those of *Emiliania huxleyi*, the most common coccolithophore species, and *Gephyrocapsa*) increases as seawater $[\text{CO}_3^{2-}]$ increases (Beaufort *et al.*, 2011). However, highly variable coccolith weight reflects extensive *E. huxleyi* genome variability, which is why this species can thrive in very diverse habitats and under a wide variety of environmental conditions (Read *et al.*, 2013).

Natural CO₂ seeps are being used to examine the response of coastal ecosystems to ocean acidification (Hall-Spencer *et al.*, 2008). Hundreds of benthic species have been investigated in these settings (Fabricius *et al.*, 2011; Porzio *et al.*, 2011; Inoue *et al.*, 2013; Kroeker *et al.*, 2013b). Meroplanktonic calcified organisms such as juvenile gastropods and foraminifera are adversely affected as CO₂ levels increase (Cigliano *et al.*, 2010), but carbon dioxide seeps have not so far been used for the study of ocean acidification on holoplankton. Here, we analyzed phytoplankton along a natural pH gradient near underwater CO₂ seeps. Several papers have been published on the acidification issue in the Levante Bay (Boatta *et al.*, 2013; Milazzo *et al.*, 2014) and show that there is a clear pH gradient in a consistent coastline direction, exactly like during our survey. We examined coccolithophore cell concentration, diversity, and coccolith morphology as well as physicochemical parameters with an emphasis on seawater carbonate chemistry. Our aims were to assess whether CO₂-driven acidification gradients revealed shifts in the diversity, abundance, and calcification of a natural coccolithophore assemblage.

Material and Methods

Seawater samples were collected on 23–26 November 2010 at eight stations along two transects running parallel to the shore off Vulcano Island in the Mediterranean Sea (Fig. 1). All stations were >200 m from a main area of CO₂ bubbling to avoid confounding factors such as the presence of H₂S (Boatta *et al.*, 2013). The methods used to count coccolithophores in water samples followed standard procedures as detailed in several recent studies (Ziveri *et al.*, 1995; Oviedo *et al.*, 2014). All samples were treated and analyzed following these procedures. For each sample, 2 l of seawater was collected in plastic or Niskin bottles from the surface 1 m of water. This was immediately passed through cellulose acetate filters (0.45- μm pore size, 47-mm diameter) using a low-pressure vacuum pump (<200 mm Hg). Each filter membrane was rinsed with distilled water buffered with ammonia after filtration to remove sea-salt, oven-dried at 40 °C, and stored in plastic petri dishes.

The samples were analyzed using polarized light microscopy for cell concentrations, and scanning electronic microscopy (SEM) for species identification and morphological examination of *E. huxleyi* specimens.

For light microscopy, a portion of filter was mounted onto a glass slide, made transparent by immersion oil, and fixed beneath a cover slip. Coccospere counts were made using a Zeiss polarized light microscope at 1000 \times . The concentration of coccospere was obtained by quantifying their number on a 5-mm² surface of the filtered area. The coccospere abundance was calculated using the following equation:

$$\text{CD} = (A \times N)/(a \times V)$$

where CD = coccospere density (number of coccospere l⁻¹); A = effective filtration area (mm²); N = total number of coccospere counted; a = analyzed area (mm²) and V = volume of filtered water (l). The methods for quantifying the species using SEM followed standard procedures (Ziveri *et al.*, 1995; Oviedo *et al.*, 2014). SEM analyses were performed by Zeiss EVO MA 10: a portion of filter membrane was mounted onto a stub for quantitative analysis of coccolith morphology of selected species in 50 coccospere and for taxonomic identification following the concepts of Young *et al.* (2003) and Jordan *et al.* (2004). A heteromorphic life cycle including diploid cells producing heterococcoliths and haploid cells holococcoliths has been described for coccolithophores (Billard and Inouye, 2004, and reference therein). Holococcolithophore species have been clustered in a single group called Holococcolithophore spp.

Temperature, salinity, and pH measurements were acquired *in situ* using a YSI 556 MPS probe with an accuracy of ± 0.1 °C, 0.1 salinity, and ± 0.05 pH. Additional 50-ml seawater samples were collected for total alkalinity analyses; these were pumped through 0.2- μm pore size filters,

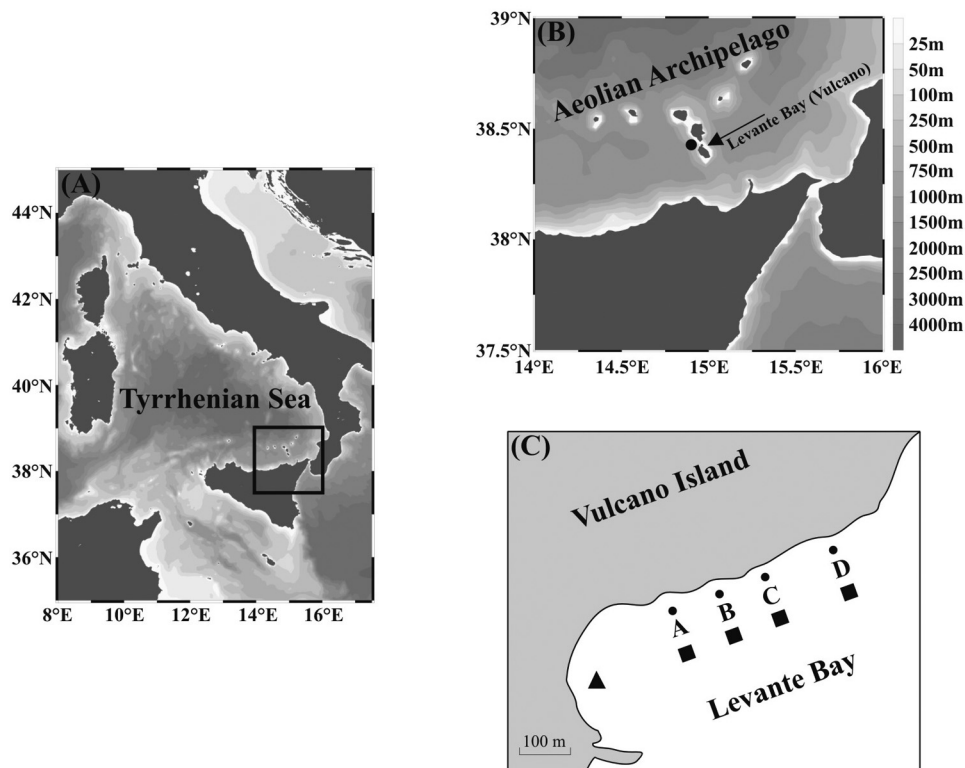


Figure 1. Bathymetry of (A) the central Mediterranean Sea and (B) the southern Tyrrhenian Sea. Black circle shows location of living coccolithophores collected in summer north of Vulcano and south of Lipari island (Bonomo, S., CNR, Consiglio Nazionale delle Ricerche, Istituto per l'Ambiente Marino Costiero, Naples, Italy unpubl. data). (C) Levante Bay, inshore (circles) and offshore (squares) sample stations and the main vent area (triangle).

poisoned with 0.05 ml of 50% HgCl_2 to avoid biological alteration, and then stored in the dark at 4 °C. Three replicate subsamples were analyzed at 25 °C using a titration system (Mettler Toledo, Inc.). The pH was measured at 0.02-ml increments of 0.1 *N* HCl. Total alkalinity was calculated from the Gran function applied to pH variations from 4.2 to 3.0, as mEq Kg^{-1} from the slope of the curve HCl volume *versus* pH. The $p\text{CO}_2$ and the saturation states of calcite and aragonite were calculated from pH_T , total alkalinity, temperature, and salinity, using the free-access CO_2 SYS package ver. 2.1 (Dickson *et al.*, 2007).

We tested for differences in the physicochemical parameters recorded on different sampling dates along the inshore transect using permutational analysis of variance (Permanova; Anderson and Ter Braak, 2003). The four sampling stations were considered as a fixed factor. The analyses were performed on Euclidian distance of untransformed data using 9999 permutations. The software Primer 6-Permanova +B20 was used to perform these analyses (Clarke and Gorley, 2006). Linear regression analyses were also run to assess relationships between pH and Ω_{calcite} (independent variables) and coccolithophore concentration, number of species, and percentage of malformed and corroded coccoliths (dependent variables).

Results

The CO_2 seeps did not affect temperature (range: 17.14–18.52 °C; Permanova, pseudo- $F_{3,8} = 0.3859$, $P = 0.7842$), total alkalinity (2516–2563 $\mu\text{mol kg}^{-1}$; Permanova, pseudo- $F_{3,8} = 2.373$, $P = 0.174$), and water salinity (37.15–37.66; Permanova, pseudo- $F_{3,8} = 0.704$, $P = 0.565$), across our inshore sampling stations (Table 1). In contrast, we recorded strong gradients in carbonate chemistry along the inshore transect; pH values increased significantly from station A (range: pH_T 6.84–7.32) at >200 m distance from the vents to station D (8.02–8.19 pH_T) farthest from the vents (Permanova, pseudo- $F_{3,8} = 10.3$, $P = 0.007$) (Table 1). There were corresponding gradients along the inshore transect for $p\text{CO}_2$ (Permanova, pseudo- $F_{3,8} = 5.398$, $P = 0.039$) and Ω_{calcite} levels (Permanova, pseudo- $F_{3,8} = 6.975$, $P = 0.033$). All the offshore stations had similar $p\text{CO}_2$ levels (400–501 μatm) and pH values (pH_T 8.00–8.08). The Ω_{calcite} was >4.6 at the farthest inshore station and at all the offshore stations, with values decreasing closer to the seeps, sometimes falling to $\Omega_{\text{calcite}} < 1$ at inshore stations A and B.

Coccolithophore concentrations ranged between 2.7×10^3 and 2.8×10^4 coccospheres l^{-1} (Table 1) and were

Table 1. Sampling sites, dates, seawater properties, and coccolithophore analysis of the studied samples

Sample	Temp (°C)	Salinity	pH _T (total scale)	pCO ₂ (μatm)	Total alkalinity (μkg ⁻¹)	Ω _{calcite} saturation state	Coccolithophore concentration (coccospheres l ⁻¹)	Number of species	<i>Emiliana huxleyi</i> (% coccospheres)	
									Malformed	Corroded
23 Nov 2010 Inshore										
A	18.43	37.41	7.32	2822.7	2526.5	1.13	7384	9	19.0	7.1
B	18.30	37.24	7.47	1963.5	2530.5	1.56	7030			
C	18.48	37.34	8.16	321.3	2535.9	6.19	9274	11	14.3	0.0
D	18.01	37.57	8.19	292.3	2516.1	6.38	14378	7		
24 Nov 2010 Inshore										
A	17.80	37.53	6.94	6971.1	2539.8	0.47	7633	5	11.4	2.3
C	18.26	37.15	7.66	1222.5	2525.3	2.33	18621	5	9.5	0.0
D	18.32	37.16	8.04	448.3	2517.9	4.94	14948	8	0.0	0.0
25 Nov 2010 Inshore										
A	17.18	37.66	6.84	8828.8	2554.3	0.37	2740	6	94.9	51.3
B	17.14	37.49	7.01	5947.1	2563.8	0.55	6180			
C	17.80	37.57	7.56	1563.0	2520.3	1.86	12909	7	9.1	0.0
D	18.28	37.47	8.02	474.7	2526.5	4.78	17011	11	5.9	0.0
26 Nov 2010 Offshore										
A	18.51	37.35	8.00	501.5	2525.9	4.63	19487	10	2.1	0.0
B	18.52	37.34	8.02	474.1	2522.7	4.80	15818	9	10.0	0.0
C	18.54	37.26	8.07	412.1	2519.0	5.26	20964	8	2.5	0.0
D	18.52	37.31	8.08	400.3	2517.1	5.35	28306	12	6.3	0.0

significantly correlated with increasing pH ($R^2 = 0.54$, $P = 0.002$, $n = 15$) (Fig. 2A), and with increasing Ω_{calcite} ($R^2 = 0.42$, $P = 0.006$, $n = 15$). Maximum cell density was usually higher at offshore stations, especially at station D (Fig. 2A). Our Nov 2010 sample from inshore station B 24 was lost, and SEM analysis was not conducted on 2 out of 15 samples (B inshore stations recovered on 23 and 25 November 2010), since inorganic nanoparticle material covered the filters, preventing taxonomic and morphological evaluation by SEM. These samples were, however, used for identification and quantification of coccospheres by polarized light microscopy. SEM observations of the D inshore 23 Nov 2010 sample allowed species identification but not detailed morphological examinations of *E. huxleyi* specimens.

We found 27 coccolithophore taxa in Levante Bay (Fig. 3). Species diversity decreased significantly as pH ($R^2 = 0.39$, $P = 0.016$, $n = 13$) (Fig. 2B) and Ω_{calcite} ($R^2 = 0.39$, $P = 0.016$, $n = 13$) fell. *Emiliana huxleyi* was always dominant although *Umbellosphaera tenuis*, *Syracosphaera molischii*, and *Syracosphaera tumularis* were major components of the coccolithophore assemblages (Figs. 3 and 4). All coccospheres of *E. huxleyi* we sampled were Type A (*sensu* Young *et al.*, 2003), being 3–4 μm wide with robust distal shield elements and curved central area elements. The diverse coccolithophore assemblages of Levante Bay (Fig. 4) are similar to those described in more open oligotrophic Mediterranean seawater settings (Knappertsbusch, 1993; Malinverno *et al.*, 2003; Bonomo *et al.*, 2012). These are the first coccolithophore assemblage data for the Tyrrhenian

Sea, but an unpublished survey between Vulcano and Lipari Islands (Fig. 1) also had a coccolithophore assemblage similar to data collected in Levante Bay in normal pH conditions: *E. huxleyi* was dominant amongst the about 30 coccolithophore species present; a few more coccolithophore species were found offshore because the assemblage included deep photic zone taxa such as *Florisphaera profunda* and *Gladiolithus flabellatus*, which were not found in the shallow waters of Levante Bay. The coccolithophore concentration at the offshore site was 3.3×10^4 coccosphere/l in the uppermost 25 m (samples collected at depths of 5 and 25 m) of the water column (Bonomo, S., CNR, Consiglio Nazionale delle Ricerche, Istituto per l'Ambiente Marino Costiero, Naples, Italy; unpubl. data).

Coccoliths and coccospheres were neither malformed nor corroded at the offshore site, whereas this was common in *E. huxleyi* specimens collected close to the CO₂ seeps (Fig. 4). Malformation was positively related to falling pH ($R^2 = 0.45$, $P = 0.016$, $n = 12$), as was dissolution ($R^2 = 0.44$, $P = 0.018$, $n = 12$) (Fig. 2C, D). However, malformation and dissolution were common only at the lowest pH and Ω_{calcite} station (Fig. 2C), and this relationship needs to be carefully interpreted. *Emiliana huxleyi* coccospheres had malformed T-shaped elements on the distal shield that were not connected with each other, were twisted, were not completely calcified, or had some combination of those malformations; while signs of dissolution were evident in the central area (Fig. 4). Some *E. huxleyi* coccospheres had corroded coccoliths at stations with low calcite saturation ($\Omega_{\text{calcite}} < 1.13$). On coccospheres with their interlocked

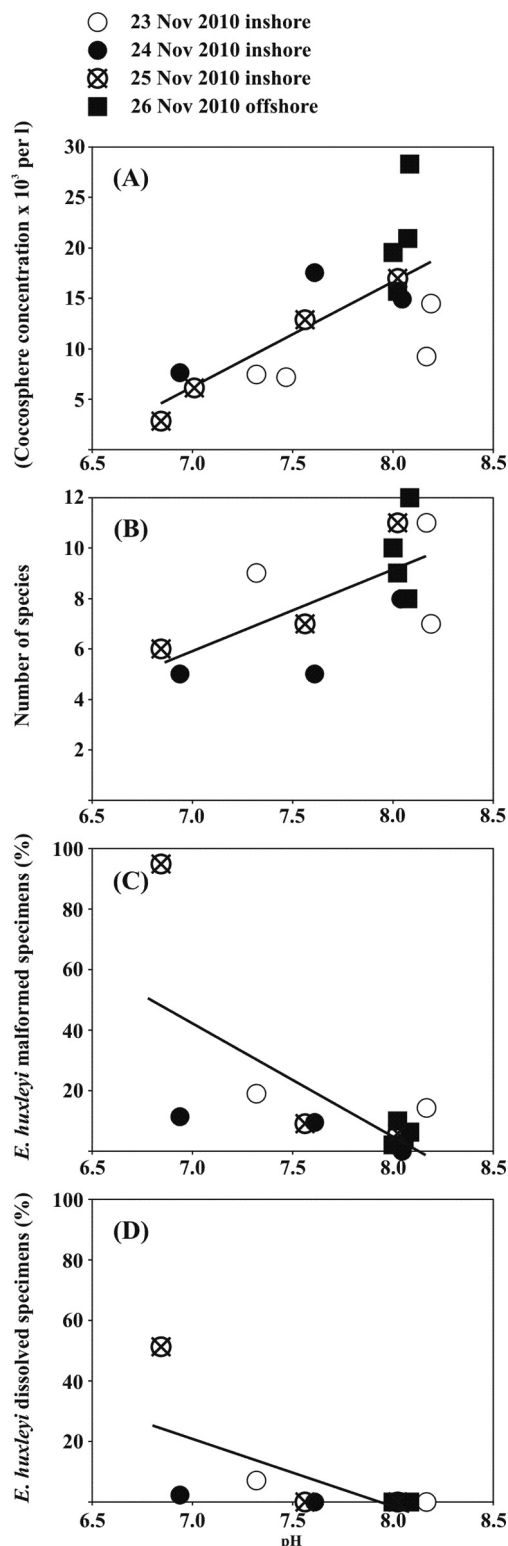


Figure 2. Coccosphere concentrations (A), species diversity (B), number of *Emiliana huxleyi* malformed (C) and corroded (D) coccoliths plotted versus pH; black, white, and crossed circles indicate samples collected from the inshore transect on 23–25 November 2010, respectively. Black squares indicate samples collected from the offshore transect on 26 November 2010. Linear regression lines are also shown.

liths still attached, the dissolution was most evident around the central area, where the central tube was partly corroded.

Discussion

Previous studies along pH gradients near Mediterranean volcanic seeps have demonstrated that although seawater acidification with CO₂ can benefit some organisms, such as heterokont algae, it is detrimental to most sessile calcified species and leads to a reduction in biodiversity (Porzio *et al.*, 2011; Johnson *et al.*, 2013; Kroeker *et al.*, 2013b). Here we show that CO₂ seeps may also be useful for studying the effects of ocean acidification on plankton. We found a diverse coccolithophore assemblage that was typical of oligotrophic conditions in the Mediterranean. Coccolithophore abundance decreased significantly with decreasing pH; water near to the seeps (pH 6.84) contained far fewer species and had malformed and corroded specimens of *E. huxleyi*. Transplant experiments along the same CO₂ gradient have shown that recruitment of reef-building gastropods was adversely affected and that exposure to acidified conditions predicted for the year 2100 and beyond caused dissolution and a significant difference in the mineralogical composition of the recruited shells (Milazzo *et al.*, 2014).

We found a progressive decrease in the diversity of coccolithophore species as CO₂ levels increased and Ω_{calcite} values fell. Coccolith malformation and dissolution occurs when pH is lowered in culture experiments (Bach *et al.*, 2012; Kottmeier *et al.*, 2014), and our results are the first evidence of this effect in natural coccolithophore assemblages. The fall in coccolithophore diversity and cell concentration pattern as CO₂ levels increased is not explained by dissolution of the assemblage at the seep. Although cell abundance and species diversity increased toward offshore and reference (D inshore) stations, at the seep site we did not detect any evidence of species selection based on lightly versus heavily calcified species. For example, delicate species of *Discosphaera tubifera*, *Syracosphaera* sp., and Holococcolithophores (Fig. 4) were present at the lowest pH stations (Table 2). Coccolithophore diversity and abundance within Levante Bay may be due to (1) slow surface currents that carry upper photic zone coccolithophores into the bay, with detrimental conditions resulting in assemblage depauperization toward the seeps; or (2) almost complete dissolution of the community at the seep and subsequent dilution further from this area, coupled with the mixing of new surface water from offshore. Future plankton surveys around CO₂ seeps would benefit from the use of current meters to monitor water residence time.

Malformed coccoliths are almost absent in coccolithophores from the Mediterranean Sea (Cros and Fortuño, 2002; Malinverno *et al.*, 2008; Oviedo *et al.*, 2014), and the presence of malformed *E. huxleyi* coccoliths at the Vulcano

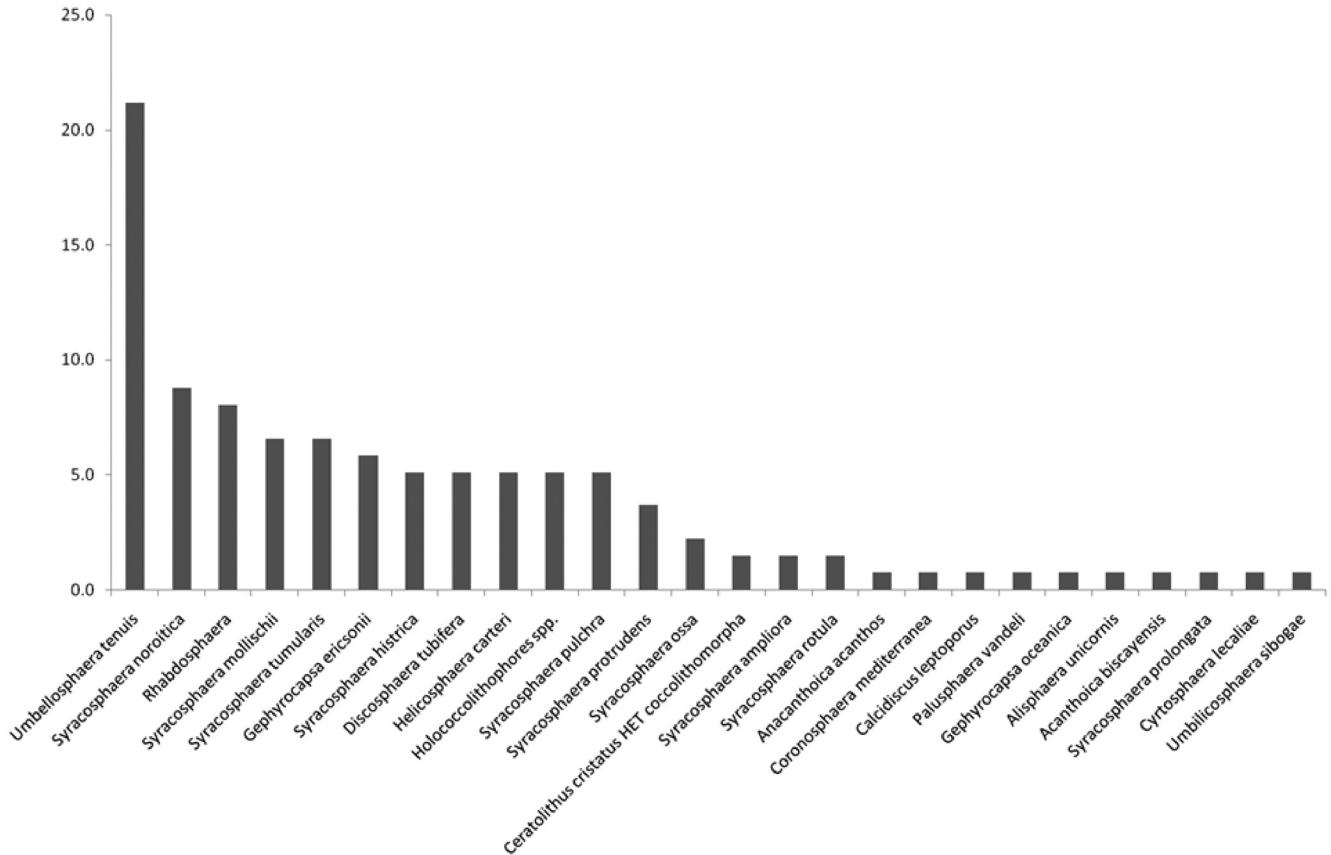


Figure 3. Percentage contribution of the diverse coccolithophore community plotted as average of the whole Levante Bay data set and ruling out the dominant (79%) species, *Emiliana huxleyi*.

seep site waters suggests anomalous environmental conditions affecting morphogenesis. The significant statistical relationship between pH values and malformed and corroded *E. huxleyi* coccoliths (Fig. 2C, D) is due to the massive malformation and dissolution in the A inshore (25NOV10) sample. Such a significant perturbation in the coccolithophore community did not occur the day before (sample A inshore 24NOV10), despite the fact that pH and carbonate chemistry seawater values were similar. Information on water residence time may explain this discrepancy. In any case, it seems that very low pH water was the primary cause of *E. huxleyi* malformation.

Strain-specific responses of *E. huxleyi* calcification rate under elevated CO₂ conditions have been reported in culture experiments and in upwelling regions, possibly due to the sensitivity of genetically different *E. huxleyi* strains to acidification (Langer *et al.*, 2009; Beaufort *et al.*, 2011; Read *et al.*, 2013). The genomic plasticity of *E. huxleyi* has recently been confirmed and may explain much of the phenotypic variation, ecological dynamics, and physiological heterogeneity observed in the geological record (Read *et al.*, 2013). Here we highlight the vulnerability of *E. huxleyi* type A specimens to lowered pH, which is expressed by reduced

cell density and abnormal morphogenesis, suggesting that H⁺ is a major factor causing malformation, as demonstrated in laboratory experiments (Bach *et al.*, 2012)

An important consideration when working near CO₂ seeps is the possible influence of confounding factors such as dissolved metals and H₂S (Hall-Spencer *et al.*, 2008). At Vulcano, rocky shore transects have been established that are not contaminated with H₂S; these can, however, have high seawater iron concentrations (Boatta *et al.*, 2013) that can affect CaCO₃ production rate in *E. huxleyi*.

The seep region had elevated levels of some trace elements in the sediments (Vizzini *et al.*, 2013). Malformed *E. huxleyi* specimens collected at high CO₂ levels had lith dissolution (Fig. 4) likely due to low carbonate saturation rather than to elevated levels of trace metals, but it would be of interest in future studies to determine whether ocean acidification can act synergistically with Fe or other metals to alter marine ecosystems, since many coastal regions have elevated trace metal concentrations.

Considerable spatial and temporal variations in CO₂ levels around the seeps make it difficult to determine dose-response relationships for pelagic organisms; this is why most work to date has focused on sessile benthic organisms

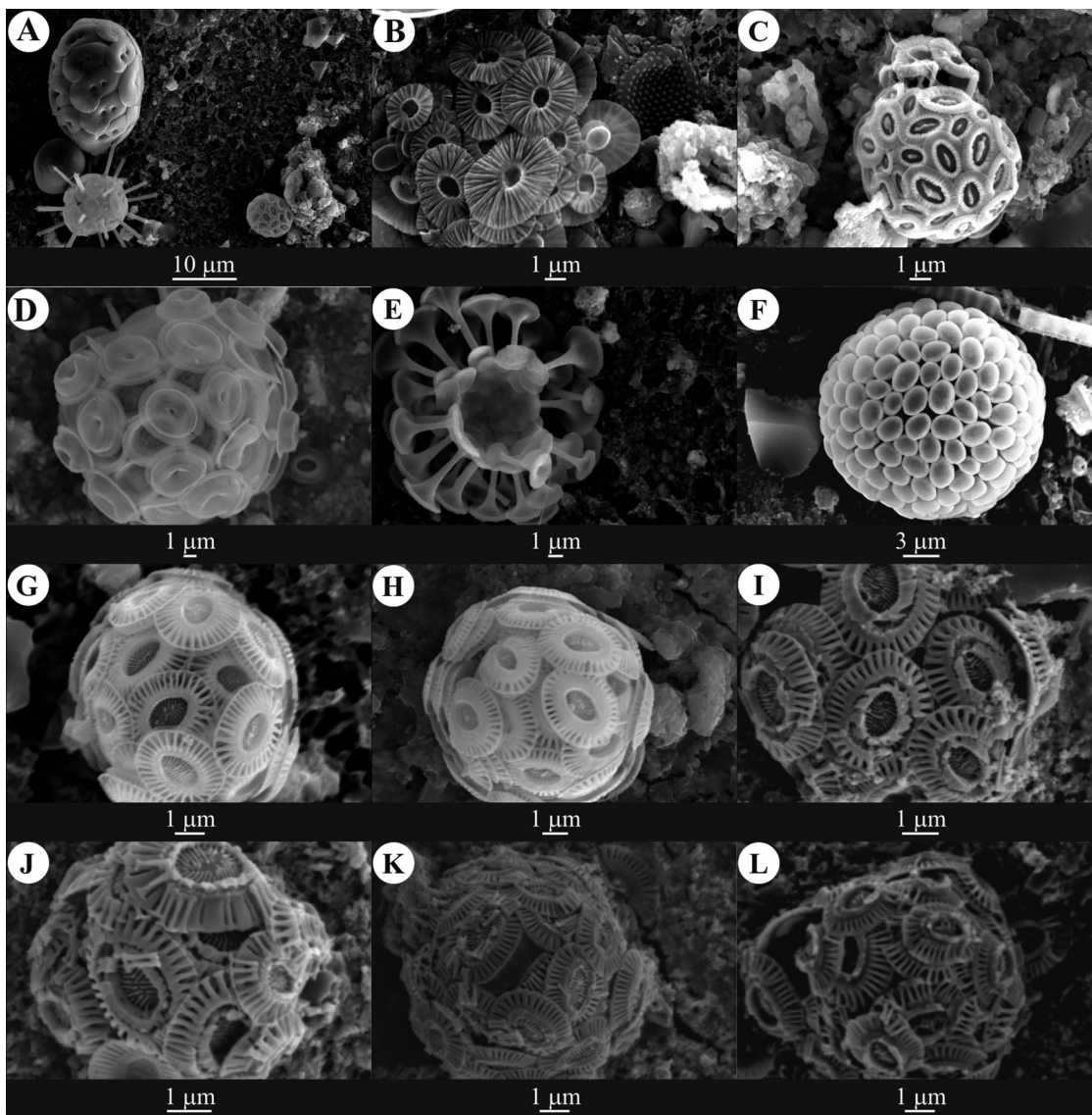


Figure 4. Scanning electron microscope images of coccolithophores collected in Levante Bay, November 2010. Coccospheres of (A) *Helicosphaera carteri*, *Rhabdosphaera clavigera*, and *Syracosphaera mollischi*; (B) *Umbellosphaera tenuis*; (C) *Syracosphaera mollischi*; (D) *Syracosphaera pulchra*; (E) *Discosphaera tubifera*; (F) *Syracosphaera pulchra* HOL *oblonga* (*Calyptosphaera oblonga*); (G) and (H) Perfectly preserved *Emiliana huxleyi*; (I)–(L) Malformed specimens of *E. huxleyi* with dissolution in the central area.

rather than fish or plankton that can move in and out of the acidified areas (Fabricius *et al.*, 2011; Rodolfo-Metalpa *et al.*, 2011; Inoue *et al.*, 2013). For phytoplankton, levels of exposure to elevated CO_2 at volcanic seeps will depend upon wind-driven currents. We found that upper photic zone coccolithophore communities in southern Tyrrhenian Sea water and Levante Bay were very similar in areas unaffected by elevated CO_2 . Future studies could combine *in situ* mesocosm-type experiments to constrain the movement of plankton at the seeps or use Free Ocean Carbon Experiments to control the CO_2 dose (Arnold *et al.*, 2012). Given the shallow nature of the seeps, such work could also

incorporate investigations of the possible effects of ultraviolet radiation.

Conclusion

Natural assemblages of living coccolithophores were investigated off Vulcano Island, southern Italy, along a pH and Ω_{calcite} gradient formed by CO_2 seeps. Coccosphere concentrations decreased significantly as pH levels fell ($R^2 = 0.54$, $n = 15$). Species diversity also declined as pH fell, with the greatest number of malformed and corroded *E. huxleyi* coccoliths nearest to the seeps where Ω_{calcite} was

Table 2. Coccolithophore species identified in the Vulcano sampling sites; pH and Ω_{calcite} are also indicated

Station	pH	Ω_{calcite}	<i>Emiliana huxleyi</i>	<i>Coronosphaera mediterranea</i>	<i>Syracosphaera mollischi</i>	<i>Rhabdosphaera clavigera</i>	<i>Umbellosphaera tenuis</i>	<i>Syracosphaera ossa</i>	<i>Syracosphaera tumularis</i>	<i>Discosphaera tubifera</i>	<i>Helicosphaera carteri</i>	<i>Syracosphaera protrudens</i>	Holococcolithophores spp.	<i>Calcidiscus leptopus</i>	<i>Syracosphaera noroîtica</i>	<i>Syracosphaera pulchra</i>	<i>Syracosphaera rotula</i>	<i>Ceratholithus cristatus</i>	HET coccoithomorpha	<i>Syracosphaera histrica</i>	<i>Gephyrocapsa ericsomii</i>	<i>Palusphaera vandeli</i>	<i>Syracosphaera prolongata</i>	<i>Cyrtosphaera lecaliae</i>	<i>Umbilicosphaera sibogae</i>	<i>Gephyrocapsa oceanica</i>	<i>Alisphaera unicomis</i>	<i>Acanthoica biscayensis</i>	<i>Syracosphaera ampliora</i>	<i>Anacanthoica acanthos</i>	
A inshore (25NOV10)	6.84	0.37	x				x		x				x	x	x																
A inshore (24NOV10)	6.94	0.47	x				x			x	x																				
A inshore (23NOV10)	7.32	1.13	x	x	x	x	x	x		x	x									x											
C inshore (25NOV10)	7.56	1.86	x			x	x							x	x			x		x											
C inshore (24NOV10)	7.66	2.33	x						x	x						x				x											
A offshore (26NOV10)	8.00	4.63	x		x				x		x		x	x						x	x	x	x								
D inshore (25NOV10)	8.02	4.78	x		x	x	x					x	x	x	x	x				x				x							
B offshore (26NOV10)	8.02	4.8	x		x	x	x			x						x				x	x				x						
D inshore (24NOV10)	8.04	4.94	x		x		x			x		x		x			x														x
C offshore (26NOV10)	8.07	5.26	x		x	x	x						x			x					x					x					
D offshore (26NOV10)	8.08	5.35	x		x	x	x	x		x	x		x							x							x	x			
C inshore (23NOV10)	8.16	6.19	x		x	x	x				x	x				x		x	x											x	x
D inshore (23NOV10)	8.19	6.38	x			x	x	x			x				x	x															

lowest. These field observations show that ocean acidification was correlated with reduced coccolithophore diversity and disruptions in coccolith morphogenesis. Further work is required to assess effects on noncalcified phytoplankton and to check the bioavailability of metals such as iron that increased in concentration near to the CO₂ seeps. Our *in situ* observations add to concerns based on laboratory and mesocosm work that calcifying phytoplankton will be adversely affected by ocean acidification.

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