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Geochemistry of two shallow CO_2 seeps in Shikine Island (Japan) and their potential for ocean acidification research

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HIGHLIGHTS

- Report on the discovery of two CO_2 seeps in the temperate Pacific Ocean, Japan.
- Geochemistry survey showed the suitability for ocean acidification research.
- Confounding factors and their mitigation for the use of CO_2 seeps in ocean acidification research is highlighted.
- Potential and past research of CO_2 seeps in Japan is discussed.

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ABSTRACT

Shallow CO_2 seeps, where CO_2 gas is venting underwater, offer great potential for studies into the effects of ocean acidification at the ecosystem level. To our knowledge, only two tropical system and two temperate systems of such seeps have been described worldwide. Here we describe two new temperate systems: the Mikama Bay and Ashitsuke sites, located on Shikine Island, Japan. The Mikama Bay site is located in a shallow bay. Investigation of the gas and water chemistry showed that the gas contained 98% CO_2 and up to 90 ppm H_2S. Total alkalinity was constant in time and space with an average of $2265 \pm 10\ \mu$ mol kg^{-1}. Mapping of Eh and pH showed that the low pH zones were the largest when currents were moderate. Under moderate currents, Eh values were globally higher and total sulfides concentration lower, supporting that a longer residence time of the bay water allow the oxidation of the sulfides to sulfates. Zones suitable for acidification studies: with a pH lower than 8.0, low saturation state of calcite and aragonite, and non-detectable sulfide concentration, can be defined a few meters from the main venting zone. The second site, Ashitsuke, is located in the inter-tidal zone on a shore composed of boulders. Several areas showed reduced pH sometimes restricted to a few meters and up to 20 m long along the shoreline. Temperature was higher in some of the reduced pH zones suggesting the presence of hot springs in addition to vents. This paper also highlights the need for discovering additional CO_2 seeps, which by their nature often lack comparable replicates and can be confounded by factors other than CO_2. In this regard, Japan offers great potential as it is home to numerous active volcanoes, representing potential venting sites in climates ranging from tropical to sub-polar.

1. Introduction

Increased atmospheric carbon dioxide (CO_2) has led to a decrease in the pH of the oceans by 0.1 in the last 100 years. Ocean pH levels are predicted to further decrease by 0.5 unit of pH in the next 100 years, a phenomenon known as ocean acidification (Doney et al., 2009). This unbuffered decrease in pH has been shown to affect a wide range of organisms in the oceans. Calcifiers, organisms that build skeletons or external shells from calcium carbonate, such as corals, foraminifera, and gastropods, are particularly sensitive to an increase in seawater pCO_2, as it lowers calcium carbonate saturation state (Cohen and Holcomb, 2009). In contrast, other organisms may benefit from increased water pCO_2, such as the

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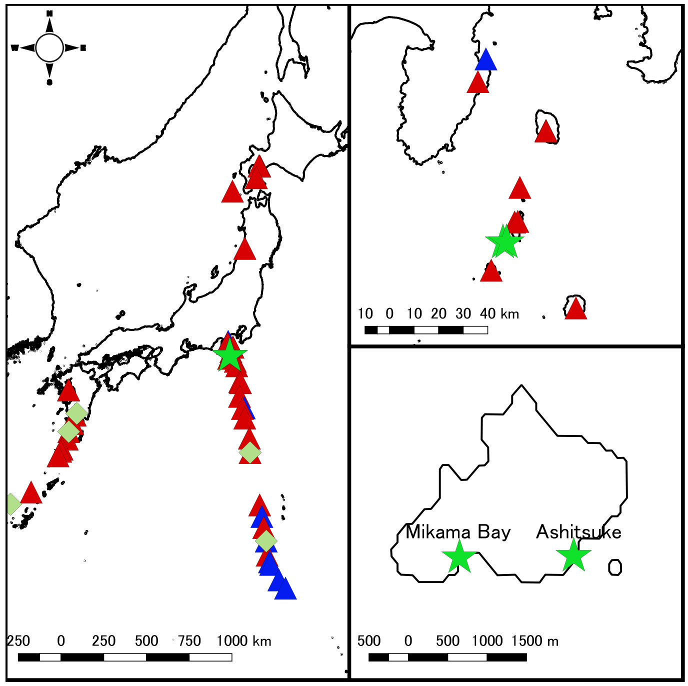

Fig. 1. Map of terrestrial volcanoes (red triangles) and submarine volcanoes (blue triangles) along the Japanese coastline, and at the two sites surveyed in this study. Insets show the Izu peninsula and the Izu Islands (top right), and Shikine Island with the Mikama Bay and Ashitsuke sites (bottom right). Shallow venting sites highlighted in Table 1 are shown (green diamonds). Location data for volcanoes are provided by the Hydrographic and Oceanographic Department of the Japan Coast Guard (http://www1.kaiho.mlit.go.jp/GIJUTSUKOKUSAI/kaiikiDB/list-2.htm, in Japanese). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

coccolithophore *Emiliania huxleyi*, which can use the additional available CO_2 for photosynthesis under certain conditions (Iglesias-Rodriguez et al., 2008), diatoms or seagrass which were shown to increase under high pCO_2 (Johnson et al., 2011). Such variability in the response of organisms to ocean acidification makes it difficult to predict the overall effects of ocean acidification at the ecosystem level. An experimental approach would require that an important number of species are studied just to have an idea of the effect of acidification on one ecosystem, and this approach would still not provide an understanding of the effects of inter-species interactions. Therefore, alternative approaches are required. Dr. Hall-Spencer et al. (2008) reported the effects of natural CO_2 vents on a coastal ecosystem near the island of Ischia, Italy, providing acidification information at the ecosystem level for the first time, and revealing the potential of CO_2 vents as a new approach to research on ocean acidification (Hall-Spencer et al., 2008).

Natural CO_2 seeps (submarine CO_2 vents) occur in various places around the world, and are generally associated with volcanic activity. After the first report by Hall-Spencer et al. (2008), other studies of such systems have been published. For example, Fabricius et al. (2011) reported the effects of increased CO_2 on a coral reef in Papua-New-Guinea, and Inoue et al. (2013) focused on the impact on a coral reef in the Ryukyus Archipelago in Japan. More recently, the ecosystems around other CO_2 seeps in New Zealand have been studied (Brinkman and Smith, 2014), and other temperate CO_2 seep ecosystems have been reported from the Mediterranean Sea (Boatta et al., 2013), near the Ischia site previously described (Hall-Spencer et al., 2008). In addition to these CO_2 seeps, other naturally acidified coral reef ecosystems have been described from Puerto Morelos, México where springs discharge low pH waters into the reefs (Crook et al., 2013, 2011). Therefore, to our knowledge, the natural acidification of only two temperate ecosystems and three tropical ecosystems, when the Puerto Morelos is included, have been studied thus far, which comprise a very small fraction of the diverse marine ecosystems on Earth.

Japan lies at the junction of four tectonic plates, and as a result is home to numerous volcanic systems, many of which are on the coastline or comprise submarine volcanoes (Fig. 1). The Japanese archipelago extends across a wide range of latitudes, 24–46°N, from tropical to sub polar zones. As such, there is a high probability of discovering marine CO_2 seeps in different climates in these regions around Japan. Deep hydrothermal vents with high CO_2 contents have been actively studied (i.e.: Ohta and Kim, 2001) but the identification and description of shallow seeps are still scarce. One critical exception to this is the shallow CO_2 seep discovered on the volcanic island of Iwotorishima in the Ryukyu arc (Inoue et al., 2013), which marked the beginning of ocean acidification research using natural vents in Japan. The Izu–Bonin–Mariana arc system is a good candidate for hosting CO_2 seeps. One of the advantages of this arc system is that it ranges from temperate to tropical zones and therefore may provide the opportunity to study the effects of ocean acidification on diverse marine ecosystems.

In this paper, we report the discovery of two CO_2 seeps in the Izu arc and present results from studies on geochemistry at these sites. We also discuss the drawbacks in using natural vents in ocean acidification research and based on our own experiences, propose ways in which these drawbacks can be mitigated. Finally, we discuss the overall potential of the Japanese arc systems for using CO_2 seeps to study the effects of ocean acidification on marine ecosystems.

2. Materials and methods

2.1. Study sites

Two different sites were investigated: Mikama Bay and Ashitsuke. Both sites are situated on the south coast of Shikine Island, off the coast of the Izu peninsula (Fig. 1). Shikine Island is located at 34°N, and it is in a temperate zone, under the influence of the Kuroshio warm current. As a result, numerous

Table 1
Chemistry data: pH, Eh, and total sulfides in the waters of Mikama Bay. Sample locations are shown in Fig. 2.

Date location	Sample ID	Latitude	Longitude	Depth (m)	Temp (°C)	Salinity	Eh (mV)	Total sulfide (nmol l^{-1})	PH	Alkalinity (μmol kg^{-1})	pCO_2 (μatm)	Ωca	Ωar
20141030													
Mikama	1	34.32022	139.20304	5.30	24.80	34.2	−1	<40	8.03	2251	596	3.74	2.46
	2	34.32016	139.20308	5.05	25.80	34.2	−64	>410	7.84	2238	976	2.55	1.68
	3	34.32045	139.20277	3.15	26.80	34.2	−47	240	7.55	2264	2 040	1.40	0.92
	4	34.32038	139.20288	3.75	27.80	34.2	−47	280	7.80	2239	1 081	2.35	1.55
	5	34.31986	139.20294	6.10	28.80	34.2	3	<40	8.06	2251	549	3.96	2.60
	6	34.31984	139.20303	9.25	29.80	34.2	−8	<40	8.06	2261	552	3.97	2.61
	7	34.31894	139.20652	6.15	30.80	34.2	73	<40	8.14	2255	442	4.60	3.03
	8	34.32063	139.20300	2.05	31.80	34.2	−45	>410	6.86	2251	10 458	0.30	0.20
20150422													
Mikama	9	34.32000	139.20340	5.70	15.65	34.5	69	<40	7.99	2272	626	2.69	1.73
	10	34.32021	139.20300	4.40	16.05	34.5	38	<40	7.78	2267	1 066	1.76	1.13
	11	34.32008	139.20360	6.70	15.70	34.5	64	<40	7.98	2272	642	2.64	1.7
	12	34.31947	139.20370	8.70	15.53	34.5	112	<40	8.12	2275	445	3.47	2.23
	13	34.31955	139.20450	8.25	15.48	34.5	134	<40	8.13	2274	433	3.54	2.27
	14	34.32061	139.20510	8.95	15.55	34.6	142	<40	8.06	2268	519	3.09	1.98
	15	34.32146	139.20460	3.15	16.20	34.5	96	<40	7.46	2271	2 333	0.89	0.57
	16	34.32150	139.20440	2.20	16.05	34.5	94	<40	7.58	2267	1 742	1.15	0.74
	17	34.32114	139.20500	7.50	15.80	34.5	69	<40	7.80	2271	1 014	1.82	1.17
	18	34.32003	139.20660	3.35	15.60	34.5	149	<40	7.96	2269	675	2.53	1.62
	19	34.32006	139.20680	45.00	15.20	34.6	133	<40	7.70	2275	1 287	1.45	0.93
	20	34.31973	139.20650	3.60	15.40	34.6	112	<40	7.97	2267	655	2.56	1.65
	21	34.32016	139.20560	8.70	15.40	34.6	155	<40	8.12	2266	442	3.45	2.22
	22	34.32069	139.20560	8.55	15.40	34.6	166	<40	8.13	2269	431	3.52	2.26
	23	34.32027	139.20360	5.55	15.70	34.6	89	<40	7.92	2275	749	2.34	1.51
	24	34.31974	139.20310	4.75	15.58	34.6	99	<40	8.04	2267	547	2.97	1.91
	25	34.31945	139.20280	3.85	15.48	34.6	125	<40	8.09	2273	481	3.28	2.11
	26	34.32007	139.20290	2.45	15.92	34.6	87	<40	7.85	2278	898	2.05	1.32
	27	34.31965	139.20330	6.75	15.50	34.6	105	<40	8.08	2279	495	3.22	2.07
20141030													
Ashitsuke													
	0m	–	–	–	22.9	24.0	–	–	7.248	2225	4 463	0.54	0.34
	2m	–	–	–	22.3	34.0	–	–	7.552	2225	1 949	1.28	0.84
	4m	–	–	–	21.4	34.1	–	–	7.999	2225	626	3.16	2.06
	6m	–	–	–	21.2	34.1	–	–	8.026	2222	582	3.31	2.15
	25m	–	–	–	21.7	33.9	–	–	7.879	2203	851	2.47	1.61
	30m	–	–	–	22.1	33.5	–	–	7.502	2203	2 210	1.13	0.73
	50m	–	–	–	20.6	34.0	–	–	8.222	2219	340	4.68	3.04

scleractinian corals and other tropical organisms can be observed in the marine ecosystem surrounding the island (Supplementary Video, Appendix A). Initial geochemical surveys were carried out to evaluate the potential of the sites before further investigations were made into water and gas chemistries.

2.2. Gas chemistry

Gas was sampled at four different locations across the venting areas in 22nd June 2014. The gas was collected in 500 ml plastic bottles by inverting a funnel above the vents (Supplementary Video 1, Appendix A). It was then transferred into a 50 ml syringe while still under water, closed with a cork, and brought onto the vessel where CO_2 and H_2S were immediately measured using 2HT (range 10%–100%) and 4LT (0.2–2 ppm) test tubes (Gastec corporation, Japan), respectively. Three samples of gas were collected in Mikama Bay and one sample was collected at the main venting site of Ashitsuke.

2.3. Water geochemistry

In Mikama Bay, the area of interest was surveyed with a multisensor (U-5000G, Horiba Ltd, Kyoto Japan) coupled with a GPS. Bottom water temperature, pH calibrated on the NBS scale, Eh, depth, and salinity were measured. Bottom water samples were collected using a Van Dorn water sampler at different locations around the bay (Fig. 2). The samples were then immediately transferred in glass DO bottles. Total sulfides were measured using a Gastec test tube for hydrogen sulfide (H_2S) (Gastec 4LT, Japan) mounted on an impinger; this occurred immediately after sampling on board the research vessel Tsukuba II. Twenty milliliters of the water samples were transferred into the impinger, 2 ml of 16 N H_2SO_4 were added, and the resulting H_2S produced was then pushed through the test tube for measurement. The remaining water sample was filtered at 0.45 μm with cellulose acetate filters (Dismic 045, Advantec Japan) and stored for total alkalinity measurements.

To assess the variability of the geochemistry in Mikama Bay the chemistry mapping and hydrology surveys was carried twice: on the 30th of October 2014 and on the 22nd of April 2015. In addition, monitoring of the variation of pH over time at a fix location was conducted by fixing a TOA DKK multisensor at a depth of 5 m close to the venting area (Fig. 2: star mark) on the 1st of May 2015. During both mapping surveys the depth, current velocity was measured at 2 and 5 m in October 2014 and April 2015 respectively using a Doppler current profiler (CI-68, FURUNO), and wind velocity were recorded using the instruments (KV-5026, KOSHIN DENKI KOGYO) aboard the research vessels Tsukuba II.

At the Ashitsuke site, pH, temperature, dissolved oxygen, salinity and Eh were measured along a 50 m transect (line 0) on the shore on the 30th October 2014 and on the 22nd April 2015 on two transect of 100 m (line 1) and 60 m (line 2) (Supplementary Fig. 1, Appendix A), using two Orion 4-Star pH-DO sensors (Thermo Scientific) equipped with an RDO probe, a pH electrode (8156BNUWP, Thermo Scientific, calibrated on the NBS scale), a conductivity electrode (Orion 013010MD, Thermo Scientific) and an ORP electrode (918BNMD, Thermo Scientific). The 0 m points are always the closest point from the same venting

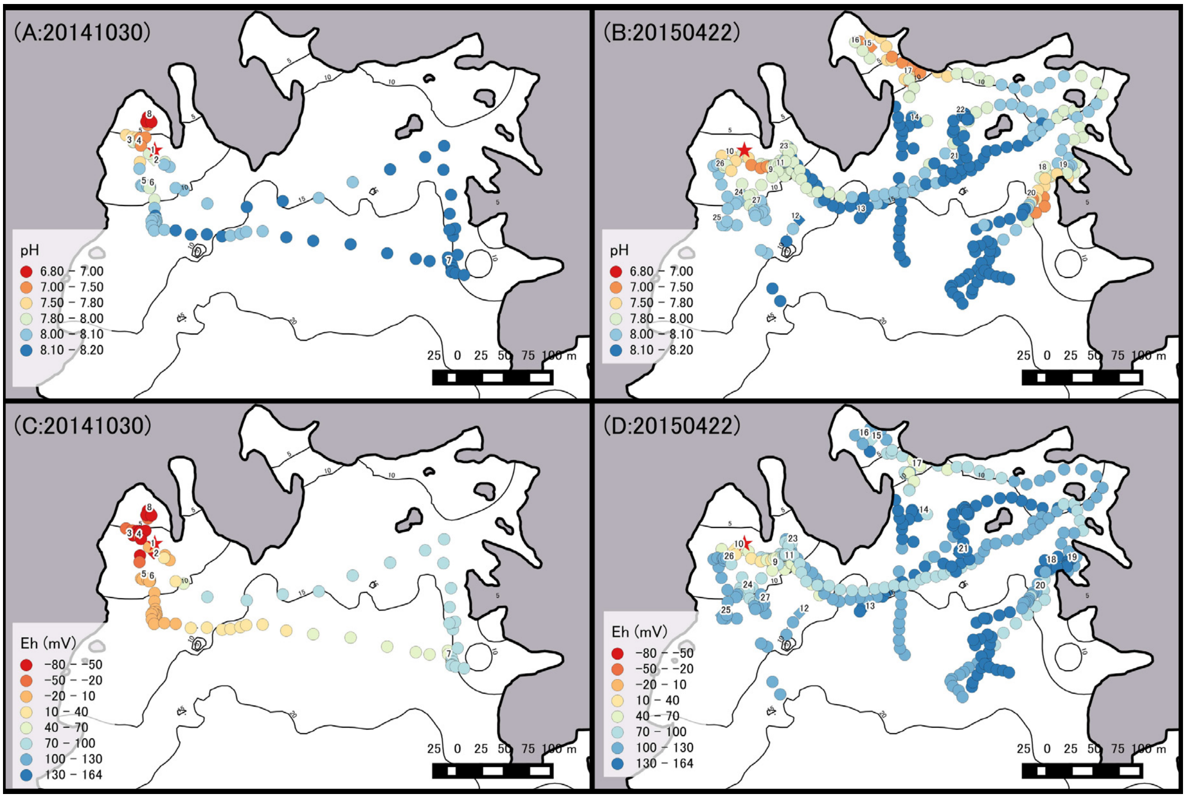

Fig. 2. pH (A: 20141030; B: 20150422) and Eh (C: 20141030; D: 20150422) in Mikama Bay. Diamonds show the location of water sampling for total sulfide measurement and total alkalinity measurements. The red starts show the location for time variation measurement of pH Contour lines show the depth in meters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

zone. Water samples for total alkalinity were taken every 5 m along line 0, on the 30th October 2014.

All water samples for total alkalinity were filtered at 0.45 μm with cellulose acetate filters (Dismic 045, Advantec Japan). Total alkalinity was measured via titration with HCl at 0.1 mol l^{-1} with a Metrohm titrator (785 DMP titrino) and total alkalinity was calculated by Gran plot from titration point with a pH between 4.0 and 3.0. Carbonate chemistry parameters: partial CO_2 pressure (pCO_2), Aragonite and calcite saturation state ($\Omega_{aragonite}$, $\Omega_{calcite}$), and dissolved inorganic carbon (DIC) were calculated using the CO2SYS software package (Pierrot et al., 2006) using pH (NBS scale) and total alkalinity with the constants from Roy et al. (1993).

Chemistry and hydrography data were plotted on maps using the free opensource software QGIS (QGIS, 2009). For vector plots (wind and currents) the plugin "Vector field renderer" (ccrook/QGIS, 0000) was used. Depth contour lines were produced in QGIS from recorded depth at random points, interpolated using the interpolation plugin (Inverse distance weighting algorithms) and corrected by hand for anomalies.

3. Results

3.1. Mikama Bay

3.1.1. Preliminary survey

The venting zone is located in a bay surrounding by cliffs, on the south coast of the Shikine Island. During our first survey in June 2014, abundant gas discharge was found in the north west of the bay, and a hot spring also discharges water nearby. The venting zone was characterized by a low pH in the water (<7), and temperature (22 °C) and salinity (34) was constant throughout the Bay. The smell of hydrogen sulfide could be detected at the surface close to the main venting site. The Bay has a steep slope towards the ocean, with depth reaching over 25 m at the entrance of the Bay (contour line in Figs. 2 and 3).

3.1.2. Gas chemistry

Gas release is very abundant at the main vents, and the vent fluxes are mostly constant (Supplementary Materials: Video 1, Appendix A). A sufficient amount of gas (500 ml) could be collected in a few seconds at three random locations in the venting zone. The gas contained 98 ± 3% CO_2 ($n = 3$). H_2S was also present, with concentrations varying from 24 to 90 ppm, with an average of 60 ± 33 ppm ($n = 3$).

3.1.3. Geochemistry

Assessment of the water chemistry in the bay showed steep pH and Eh gradients. During the survey in October 2014, the lowest pH recorded, directly above the vents, was 6.86, which corresponded to an Eh value of −70 mV (Fig. 2(A), (C)). In April 2015, three zones with reduced pH were observed (Fig. 2(B), (D)). At the first zone, corresponding to the zone found in 2014, the minimum pH observed was 7.34 with an Eh of 32 mV. At the second zone, in a small bay in the center of Mikama Bay, the minimum pH observed was 7.27 with an Eh of 67 mV. Finally, at the third venting zone on the west of Mikama Bay the minimum observed pH was 7.33 with an Eh of 85 mV. Zones with a pH of approximately 7.9 and a slightly positive Eh were observed to the east of the venting site in October 2014. In April 2015 survey, all Eh values were positive. Salinity and temperature were similar between the venting area and the remainder of the bay, with averages of 35.2 ± 0.2 and 25.1 ± 0.2 °C in October 2014, and 34.6 ± 0.1 and 15.6 ± 0.3 °C in April 2015. Total sulfide concentrations in the water varied with Eh levels. In locations and time of sampling, where Eh values were positive or close to zero, total sulfides were below the detection limit (<40 nmol l^{-1}), as it was the case for all samples in April 2015,

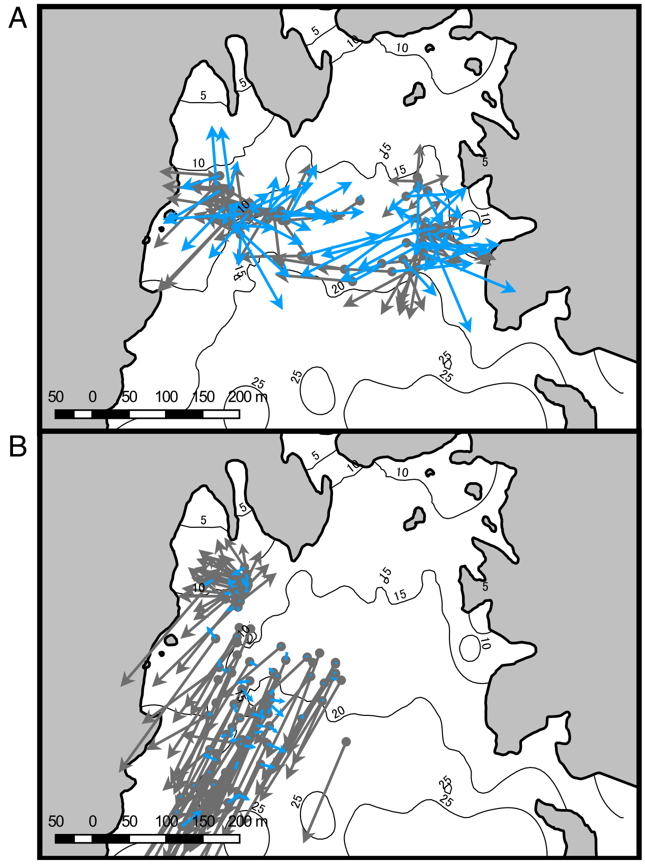

Fig. 3. Currents (blue arrows) and wind (gray arrows) recorded in Mikama Bay during the surveys on the 30th of October 2014 and 22nd April 2015. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

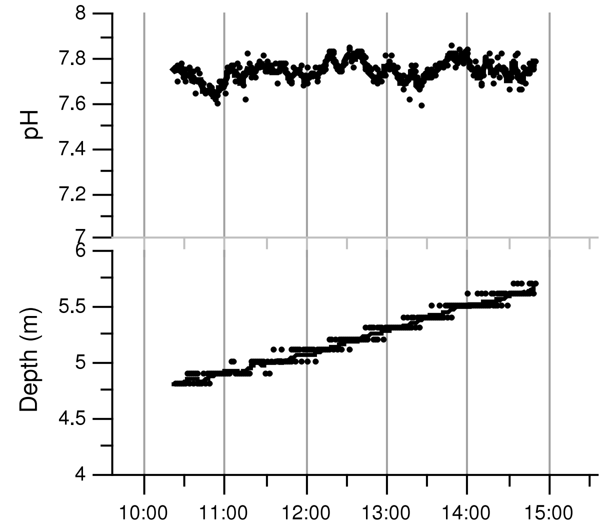

Fig. 4. Variation in pH and depth at a fix location in Mikama Bay on the 1st of May 2015.

and where Eh values were negative, concentrations varied from 240nmol l^{-1} to more than 410 nmol l^{-1} (Table 1). Total Alkalinity was relatively constant in time and space with an average value of 2265 ± 10 µmol kg^{-1}. Calculated carbonate parameters of water samples taken, showed maximum observed pCO_2 of 10,458 µatm in October 2014 and 2333 µatm in April 2015 corresponding to undersaturated value with regard to calcite and aragonite (Table 1).

Moderate turbulent winds, ranging 0.6–11.5 m s^{-1}, average 4.5 m s^{-1}, were observed in October 2014, which were associated with current velocities at 5 m in the surface water ranging from 0 to 1.6 knot, average 0.4 knot, during the surveys (Fig. 3(A)). Moderate North–North–East winds, ranging 1.5–8.6 m s^{-1}, average 5.1 m s^{-1}, were recorded in April 2015, and associated with low current velocities, ranging from 0 to 0.2 knot, average 0.04 knot (Fig. 3(B)).

Logging of pH which was conducted every minute during 3 h in May 2015 (Fig. 4) showed an average pH of 7.75 ± 0.05, ranging 7.59–7.88. During the 3 h, the depth increased from 4.7 to 5.7 m, but the variation in pH were not associated with the change of depth on the observed period.

3.2. Ashitsuke

3.2.1. Preliminary survey

The site is located on the shore near to the Ashitsuke harbor (Supplementary Fig. 1, Appendix A). The shore is composed of rocks varying in size, with the largest more than 2 m in diameter. At low tide, gas seeps can be seen in tidal pools, and hot spring water is also discharged into several pools. At high tide, the seeps are beneath the water.

3.2.2. Gas chemistry

The volume of gas being vented is not as important as in Mikama Bay and is mostly intermittent. Therefore, only one sample was collected. The gas contained 56% CO_2 and 3.3 ppm H_2S.

3.2.3. Geochemistry

Water chemistry was measured at high tide when the seeps are covered by water (Figs. 5 and 6). During the survey in 2014, a very steep gradient in pH was observed between 0 m (closest from the seep) and 5 m along the transect, with the lowest value of 7.25 at 0 m. Another drop in pH was observed at approximately 25 m, which appears to be caused by another spring. At 0 m, a decreased salinity (24), slightly increased temperature (22.9 °C) and increased Eh (121.9 mV) were recorded, but these values returned to a more typical level, either 1 m from the seep, for a salinity of 34 and Eh of 53.3 mV, or 5 m from the seep for a temperature of 21.2 °C. A similar trend, but with a smaller amplitude, was also observed at approximately 25 m. The alkalinity was mainly constant along the transect with a value of 2220 ± 7 µmol kg^{-1}. The corresponding carbonate parameters: pCO_2, $\Omega_{calcite}$ and $\Omega_{aragonite}$ ranged from 340 µatm (50 m) to 4463 µatm (0 m), 0.54–4.68 and 0.34–3.04, respectively (Table 1).

During the 2015 surveys, several low pH zones were observed in both north (Fig. 7A, Supplementary Fig. 1: line 1) and south transects (Fig. 7B, Supplementary Fig. 1: line 2) with minimum observed pH of 6.65 and 7.60 respectively. The decrease of pH was associated with an increase of temperature reaching up to 22 °C at 23 m of the north transect.

4. Discussion

The results presented here from the Mikama Bay and Ashitsuke sites highlight the potential of these sites for the study of acidification at the ecosystem level with zones naturally enriched in CO_2. The presence of potentially confounding factors is acknowledged, but if study methodologies are well planned and these factors are mitigated, then these sites can provide valuable information on the effects of acidification at ecosystem levels. The confounding factors found in this study were the presence of H_2S in the venting gas at Mikama Bay, with concentrations up to 192 ppm, and the limited spatial range of the pH gradient and increased temperature in Ashitsuke. These factors are not unique to these sites, and are often present in other natural CO_2 seep systems, potentially also having an effect on the ecosystems around the

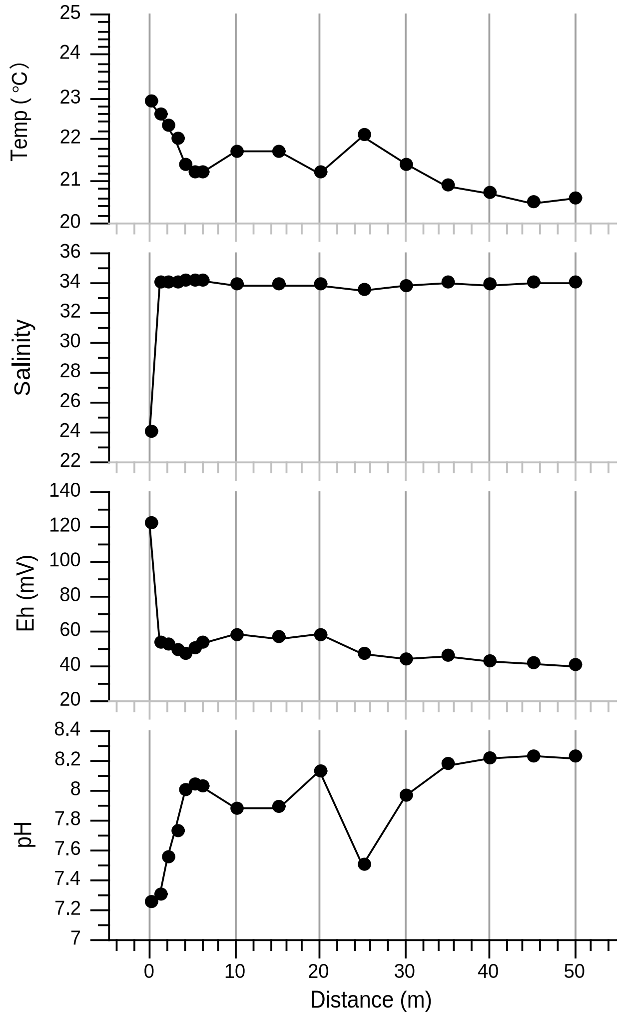

Fig. 5. Chemistry of seawater at high tide along a 50 m transect (line 0) in Ashitsuke in October 2014. Temperature, salinity, pH and Eh were measured every 1 m.

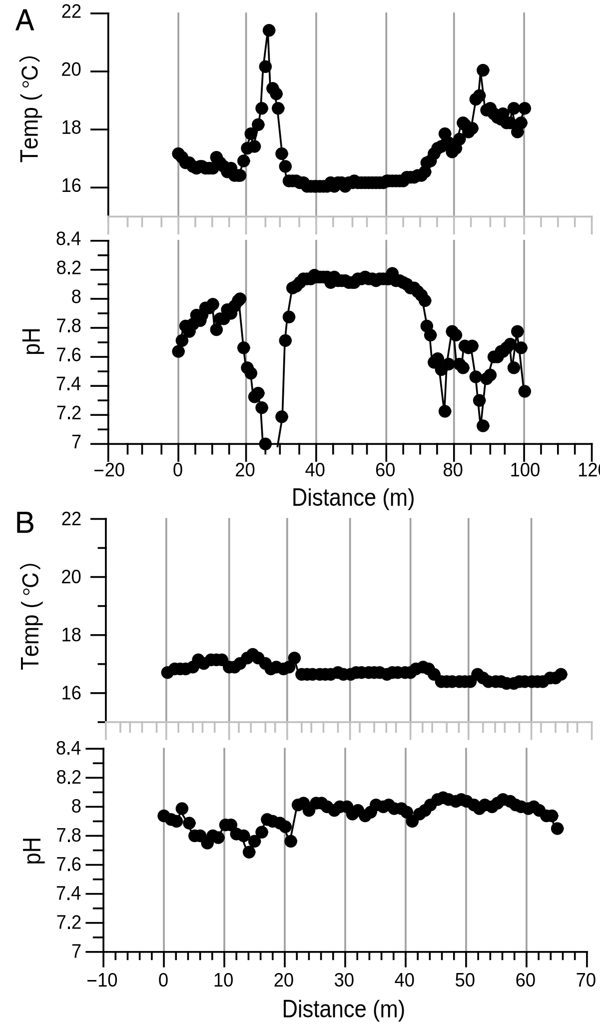

Fig. 6. Temperature and pH measured along the north and south transect (line 1 and 2 respectively). Temperature and pH were measured every 1 m.

sites (Riebesell et al., 2010; Barry et al., 2010). In order to allow for and mitigate the effects of these factors, different approaches are proposed and discussed below.

The first potentially confounding factor often associated with volcanic vents is the release of toxic gases such as hydrogen sulfide (H_2S). In seawater, H_2S dissociates into the hydrosulfide ion (HS^-) and the bisulfide ion (S^{2-}). These three species are toxic and are referred to as the total sulfides. In water containing a sufficient amount of oxygen, and with an appropriate pH, temperature and Eh, sulfides will be oxidized to diverse forms including, at the usual oxygen concentrations of surface water, non-toxic sulfate ions (Bagarinao, 1992). The venting gas at Mikama Bay contained a measurable amount of H_2S, the Eh values close to the venting site were low in one of the survey, and a relatively high concentration of total sulfides was recorded here, showing that a non-negligible portion of the H_2S released remained in the form of sulfides in the water in that region. However, at greater distances from the venting sites, Eh values became positive and no total sulfides could be detected, suggesting that most of the released H_2S had been oxidized to sulfate. During the 2015 surveys, Eh values were globally higher than during the 2014 surveys and the zones where pH was affected tended to be larger. This difference could be attributed to slowlier current at the time of the 2015 surveys (Fig. 3) and therefore a longer turn over of the water in the Bay. The oxidation of sulfide is rapid, with a half time ranging from 0.4 h to 65 h in air-saturated seawater (Bagarinao, 1992; Millero, 1986), and a high residence time of the water will allow the complete oxidation of sulfides to sulfate on a short distance range around the vent. The toxicity of sulfides to marine organisms is still not well understood, and toxic concentrations are not well constrained. However, sulfides in water solution were found to be toxic to mammals at a concentration of 2 μmol l^{-1}, while negative effects on fish physiology can be seen at concentrations greater than 0.45 μmol l^{-1} H_2S, and concentrations of 280 μmol l^{-1} were found to cause hepatic malfunctions in the freshwater common carp. Some exceptions to this general pattern exist, however, with some fish species tolerant to concentrations as high as 300 μmol l^{-1} (Tobler et al., 2006). The effect of sulfides on algae is very variable, with some thriving in high concentrations of 250–500 μmol l^{-1} and other inhibited by sulfide concentrations of 30–60 μmol l^{-1} (Bagarinao, 1992). The seagrass *Thalassia testudinum* was shown to be tolerant to underground exposure of sulfide at mmol l^{-1} levels (Koch and Erskine, 2001). Only limited literature is available on the toxicity of sulfides for marine invertebrates. Toxic sulfide levels are suggested to be 200 μmol l^{-1} and higher (Völkel and Grieshaber, 1995), but often only meiofauna and deep-sea thermal vent organisms have been considered (Thiermann et al., 2000), and other benthic and planktonic invertebrates that are not typically exposed to high sulfide concentrations may have a lower tolerance. The concentrations of H_2S and total sulfides found in Mikama Bay are comparable to those of some other natural CO_2 seeps that have been used for acidification studies. Venting gas at the Ischia system of Hall-Spencer et al. (2008), the system on Iwotorishima Island (Inoue et al., 2013) and two of the sites in Papua New Guinea (Fabricius et al., 2011) are reported to contain undetectable amounts of H_2S. In contrast, a site on Vulcano Island in Sicily, and the Dobu site in Papua New Guinea contained measurable amounts of H_2S. At Dobu, 163 ppm H_2S was measured in the venting gas (Fabricius et al., 2011). The chemistry at the Vulcano Island system in Levante Bay is well described, and data from 1984 are available (Boatta et al., 2013), showing that H_2S concentrations in the venting gas are highly variable in both space and time, with concentrations ranging from 21,600 ppm to less than 50 ppm in nearby sites. Eh and total sulfides were also measured at the Levante system, and showed a similar trend to that in Mikama Bay, with the most extreme values above the venting sites. Further from the vents, Eh and pH levels were higher, reaching levels at which sulfates will dominate (Boatta et al., 2013). The concentrations found in Mikama Bay, are well below the toxic concentrations reported in the literature, except for directly above the vents where the total sulfide concentrations were higher than 410 nmol l^{-1}. At positive Eh values most of the sulfide will be oxidized, and total sulfide concentrations of less than 40 nmol l^{-1} were found at Eh levels higher than −8 mV. This relationship provides us with a method of ensuring that the potential effects of H_2S in venting gas are mitigated. With a simple mapping of Eh and pH levels, it is possible to select zones where a decrease in pH is still present because of the high concentration in CO_2 in the venting gas, but where most of the H_2S has been oxidized to non-toxic sulfates, as indicated by positive Eh values. To confirm the suitability of the study zone, long-term measurements of pH and Eh are required, as the chemistry around venting sites was shown to be highly variable and will be strongly dependent on the current velocity and water residence time.

The spatial and temporal variability of the water chemistry around venting sites is not a good representation of future changes expected because of an increase in atmospheric CO_2 (Kerrison et al., 2011). However it has been suggested that acidification due to increased atmospheric CO_2 will also be accompanied by increase pH variation both at the organism scale (Agostini et al., 2013) and ecosystem level (Anthony et al., 2011; Schulz and Riebesell, 2012) and therefore the study of effects of rapid pH variation as seen in CO_2 seeps, on the organisms physiology may also be useful (Dufault et al., 2012). The variability of pH at venting systems is mainly determined by two different factors: the amount of gas released, and the water circulation. Water circulation will not only determine the amount of gas that will dissolve in the water mass passing the venting zone, but also the oxidation of H_2S and the release of CO_2 into the atmosphere. All the venting systems that have been studied have shown significant variation in pH over time, with a variation of up to 1 unit of pH (Hall-Spencer et al., 2008; Fabricius et al., 2011; Kerrison et al., 2011). Mikama Bay is not different and variations of 0.5 units were observed during a 3 h monitoring of pH. Variations are often coupled with the tidal cycle (Inoue et al., 2013) however this could not be observed in our study as it would require longer monitoring. The effects of the tidal cycle can be seen on two levels. Firstly, it has been shown that changing water pressure as a result of a change in depth directly affects the amount of gas released (Furushima et al., 2009). Secondly, the tidal cycle will also affect the residence time of the water, particularly in coral reefs where water exchange with the open ocean is highly reduced at low tides. The residency of water and the currents in the venting zone will also determine the steepness of the gradient. In Mikama Bay, a sharp gradient was observed over less than 100 m on the west of the Bay in October 2014 and larger (over around 200 m) in April 2015, which is more distinct than at other venting systems where the pH gradient and affected zones span several hundreds of meters (Boatta et al., 2013). The steepness of the gradient could be caused by the bay structure, including its depth which rapidly increased going south and its opening to the ocean waters. In Ashitsuke the gradient is even steeper than in Mikama bay, with pH returning to values greater than 8 over only a few meters. At this site, the seeps occur within the inter-tidal zone, and therefore, at high tide they are covered by highly active water with a high pH, limiting the zone in which pH can be affected. The pH at Ashitsuke is also in consequence expected to be highly variable. Long term monitoring of the pH in the inter tidal zone will be required for further investigation. The study of natural venting sites has been criticized owing to the fact that the community inhabiting the venting sites may have come from far away and therefore, may not reflect an adapted community that would form under the predicted future acidification caused by an increase in atmospheric CO_2 over the next hundred years (Barry et al., 2010). To mitigate the effects of potentially "temporary" residents of the venting sites, future studies could focus on non-motile organisms. Moreover, special focus should be given to the "winners", the dominant species in the low pH zones, as they likely show a natural adaptation to the low pH values, and therefore may represent the future dominant species. Finally, observations made during field studies, especially of physiological effects, should be confirmed through experiments in a controlled environment when possible (i.e. Inoue et al., 2013). Addressing fluctuations in pH over time is more problematic. Fluctuations observed at venting sites does not clearly represent long-term changes as a result of atmospheric change. Careful mapping of the water chemistry and data collection over an extended period of time can highlight areas where these fluctuations have a smaller amplitude, possibly further from the venting sites (Kerrison et al., 2011) Laboratory experiments, where fluctuations in pH can be controlled and limited, should be used to confirm the effects observed in the field.

The reduction in pH in Ashitsuke was often associated with a higher temperature. Several hot springs are present close to the seeping zones. These hot springs are mainly hot seawater and no changes in alkalinity was observed. A hot springs is also present in Mikama Bay (north west shore) but no increased in temperature was detected probably due to the large volume of water in the bay and the distance of the studied zone from the hot spring. The temperature in Ashitsuke were lower than in Mikama Bay during the October 2014 survey. This difference could be attributed to the cooling by air. The local increase in temperature in Ashitsuke will limit its use for pure ocean acidification studies but could be used to study the simultaneous effect of increased temperature and reduced pH. Sites along the south transect showed temperature increased by less than 1 °C and reduced pH that correspond to the predicted value for 2100.

Finally, because of the nature of their formation, it is often difficult to find comparable replicates of venting sites. In many cases, nearby venting sites can be identified, and often, two (Hall-Spencer et al., 2008; Brinkman and Smith, 2014) or three venting sites (Fabricius et al., 2011) have been combined in CO_2 seep studies. In other cases only one venting site of comparable characteristics could be found in an area (Inoue et al., 2013). In Mikama Bay at least two reduced pH zones were observed and could be used as replicates. However due to their proximity, their independence can be discussed and they do not represent the overall diversity of ecosystem and geomorphology that can be observed in Shikine Island. Due to the lack of replication and the complexity inherent in interpreting the data given the various potential confounding factors, it is difficult to consider ecosystems close to CO_2 seeps as a window into future ocean acidification effects on the diverse marine ecosystems. To mitigate the effects of this lack of replication, two different approaches are possible. Firstly, the statistical analysis carried out on any obtained data should be rigorously selected. Havenhand et al. (2010) suggested

Table 2
Japanese seeps reported in literature for which the gas composition is known. The seeps are potentially suitable for the study of the effects of acidification at the ecosystem level.

Venting site	Gas composition		Lat	Long	Depth (m)	
Funka-asane	CO_2	95.1%	25°27′20″ N	141°14′20″ E	20	Hydrographic Department, Maritime Safety Agency (1994)
	H_2S	4.18%				
	Others	0.7%				
Taketomi[a]	CO_2	0.45–1.9%	24°17′12″ N	123°52′54″ E	20	Noguchi et al. (2006)
	CH_4	68.2–69.5%				
	N_2	30.4–34.1%				
	O_2	0.7–1.3%				
	H_2S	0.008–0.015%				
	He	<0.0006%				
Backarc Basin	CO_2	86%–92%	27°16′54″ N	127°4′48″ E	1300–1550	Sakai (1994)
	H_2S	3–5.5%				
	Others	3.5–11				
Ogasawara arc	CO_2	3.92–149.8 mM/kg	29°47′39″ N	140°20′31″ E	692–2300	Sakai (1994)
	NH_4	0–5.59 mM/kg				
	H_2S	1.2–9.66 mM/kg				
	Others	0.5–11.0 mM/kg				
Wakamiko Caldera	CO_2	50.7–92.6%	31°39′2″ N	130°45′09″ E	75–200	Shitashima (2009)
	H_2S	<1.37%				
	H_2	<0.72%				
	N_2	<10.8%				
	CH_4	<26.5%				
	O_2	<3.22%				
	Ar	<0.157%				
Satsuma Iwojima	CO_2	>98%	30°47′35″ N	130°18′19″ E		Sato et al. (2014)

[a] Low CO_2 content in the Taketomi site is not suitable for acidification research but has provided valuable information on the dynamics and physics of venting.

a statistical methodology suitable for the study of CO_2 seeps at the ecosystem level. They proposed that the response of the ecosystem obtained at the venting sites, should be compared with the average response observed at various nearby control sites. An alternative approach is to confirm the ecological effects observed at a venting site by comparing them with the ecological effects observed at different seeps located at similar latitudes and in similar environments. Such "pseudo-replication" adds extra weight to the need of the findings of CO_2 seep studies. However, the number of seeps that have been described is still too low, with only two in temperate zones and two in tropical zones, or three if the Mexican site is included. Here, we report one more temperate shallow site at Mikama Bay, and one intertidal site at Ashitsuke. The likelihood of finding other CO_2 seeps in Japan is high, considering the number of active volcanoes along the Japanese coastline (Fig. 1).

Japan is already well known for its studies of deep hydrothermal vent (Sakai et al., 1990; Sakai, 1994; Shitashima, 2009) but a simple review of the literature points to more shallow and epipelagic venting systems (Shitashima et al., 2008; Kikawada et al., 2005; Noguchi et al., 2006; Hydrographic Department, Maritime Safety Agency, 1994) that could be used for studying ocean acidification. Some of these potential locations and their gas chemistry are summarized in Table 2. Among these, the "Wakamiko Caldera" system has already been used to assess the effect of CO_2 sequestration in marine sediments (Shitashima et al., 2008). Japan extends across a wide range of latitudes, and the Izu–Bonin–Mariana arc in particular, which hosts the sites reported in this study, has a significant number of volcanoes that are possibly associated with venting sites, in climates ranging from temperate to tropical. Combining the wide latitude range of Japan and the abundance of volcanoes, Japan has the potential to host a wide varieties of CO_2 seep systems and therefore could afford researchers great opportunities for ocean acidification research using naturally CO_2 enriched systems. Our future studies will continue to focus on investigating suitable sites along the Izu–Bonin arc.

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Appendix A. Supplementary materials

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