

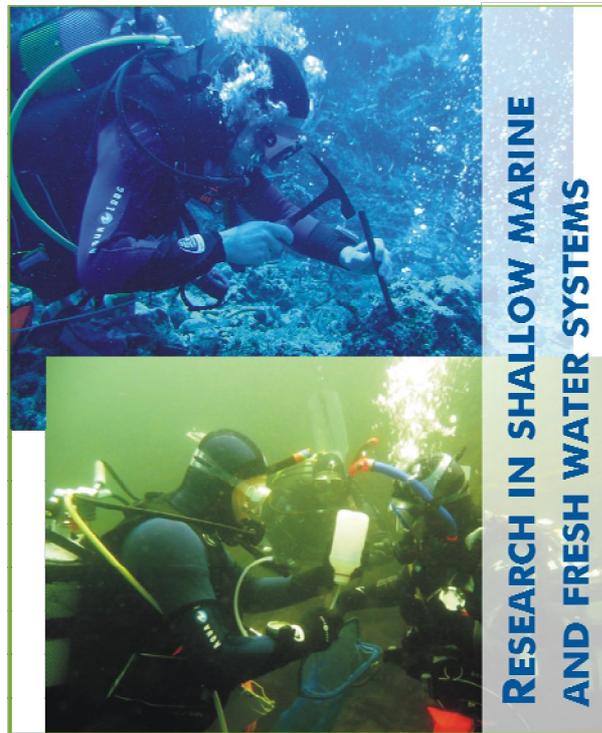
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# Using scientific diving to investigate the long-term effects of ocean acidification at CO<sub>2</sub> vents.

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**Abstract.** We are using SCUBA to document ecosystem responses to long-term ocean acidification at volcanic CO<sub>2</sub> vents. There are 30% reductions in biodiversity at average pH 7.8-7.9 (minimum pH 7.4), compared with areas with normal pH (8.1-8.2). Some groups (seagrasses and many algae) are tolerant of the increased CO<sub>2</sub> levels but others (corals, sea urchins and calcified algae) are removed from the ecosystem. Transplant experiments show dissolution of calcareous organisms and our study demonstrates, for the first time, what happens to coastal marine ecosystems when key groups of species are killed due to rising CO<sub>2</sub> levels.

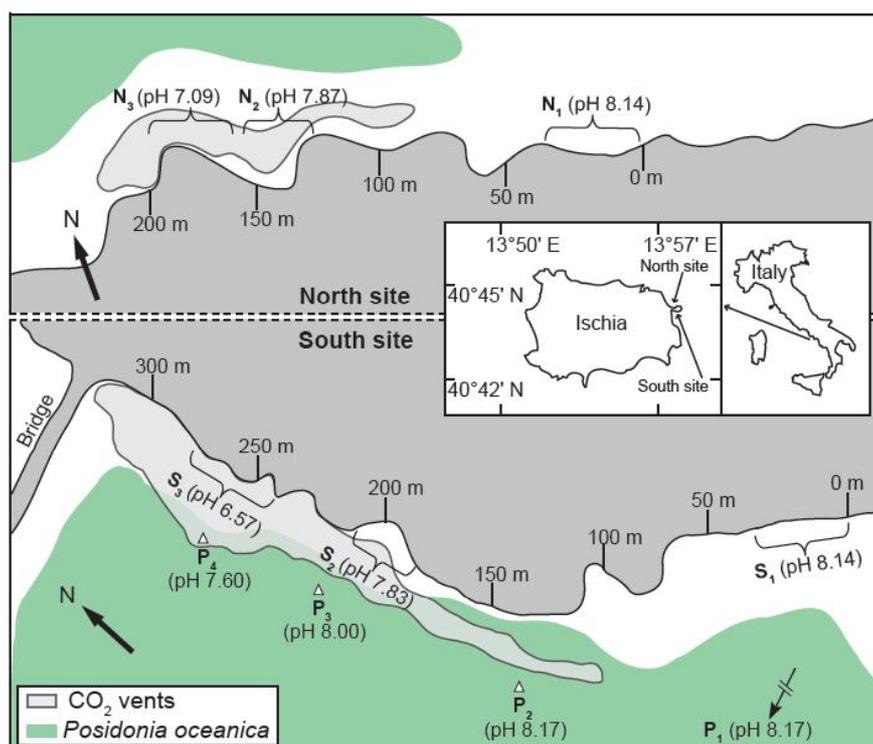
## Introduction

The global oceans currently absorb over 25 million tons of CO<sub>2</sub> every day. This has caused surface waters to become 30% more acidic since wide-spread burning of fossil fuels began. As well as lowering pH, increased CO<sub>2</sub> levels are altering surface water chemistry, causing a decline in carbonate ions, an increase in bicarbonate ions and lowering calcium carbonate saturation states. Falling calcite and aragonite levels are a concern since these are the building-blocks of shells for a range of marine organisms from tiny coccolithophores to giant coral reefs. Research into the marine environmental effects of increased oceanic CO<sub>2</sub> levels is mainly being carried out using short-term-shock experiments whereby pH or CO<sub>2</sub> levels are manipulated in aquaria and enclosures over short timescales (Hall-Spencer et al. 2008).

To examine long-term ecosystem effects of ocean acidification researchers have begun to exploit natural gradients in pCO<sub>2</sub>, such as differences in plankton community composition in the Baltic vs. Black Seas (Tyrrell et al. 2008), the depth distribution of cold-water corals in relation to the aragonite and calcite saturation horizons (Guinotte et al. 2006), tropical coral calcification strength in the high carbonate Caribbean vs the low carbonate Galapagos (Manzello et al. 2008), or changes in gastropod shell length and dissolution along gradients of estuarine pH (Marshall et al. 2008). Deep sea vents are also usually acidified, which may explain their characteristic lack of echinoderms (Van Dover 2000), and recent studies show that CO<sub>2</sub>-rich vents in the Okinawa Trough indicate strong coupling of faunal patterns and pH (Boetius et al. unpublished). Our ongoing research programme is utilizing natural gradients in pH to investigate the effects of ocean acidification at coastal CO<sub>2</sub> vent sites. We have begun with comparisons of community structure and the condition of individual species along these gradients providing strong evidence that environmental hypercapnia has both negative and positive effects on the local species pool (Hall-Spencer et al. 2008, Martin et al. 2008).

## Methods

Vents are being studied on the north and south sides of Castello Aragonese, Ischia, Italy (40°043.84'N; 013° t to steeply sloping rocky shores (Fig. 1).



**Fig. 1.** Map of CO<sub>2</sub> vent sites north and south of Castello Aragonese. Mean surface pH is shown at 35-m wide stations N1-N3 and S1-S3. Mean subtidal pH is shown at stations P1-P4 together with distributions of the gas vents and sea-grass meadow.

### Physical measurements

Gas emission is monitored using SCUBA divers who deploy 1 m<sup>2</sup> plastic funnels connected to 250 ml bottles placed on the seabed in areas of high (>10 vents m<sup>-2</sup>, constant flow), medium (5-10 vents m<sup>-2</sup>, constant flow) and low (<5 vents m<sup>-2</sup>, intermittent flow) gas emission rates. There is a video of this at

<http://news.bbc.co.uk/2/hi/science/nature/7437862.stm>.

Gas is collected for analysis in evacuated 50 ml glass tubes, partially filled with 20 ml of 0.1M Cd(OH)<sub>2</sub> + 4N NaOH suspension and connected to a plastic funnel positioned over the rising bubbles. A YSI/25 FT pH (NBS scale) meter is used for rapid assessment of salinity, temperature and pH and surface and bottom water samples are taken for more precise measurements of the spatial and temporal variability in pH (in total scale) measured immediately after water sample collection using a meter accurate to 0.01 pH units (Ion 6, Acorn Series, EUTECH Instruments, Singapore) and calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions. Irradiance and temperature are monitored using Hobo Onset Computer® data loggers.

Seawater samples are passed through 0.45 µm pore size filters (GF/F Whatman) and poisoned with 0.05 ml of 50% HgCl<sub>2</sub> (Merck, Analar), to avoid biological alteration, then stored in the dark at 4°C. A titration system composed of a pH meter with an ORION pH electrode (calibrated using NBS standard solutions) and a 1 ml automatic burette (METHROM) is used to analyse 20 ml samples at 25°C. The pH is measured at 0.02 ml increments of 0.1 N HCl. Total alkalinity is calculated from the Gran function applied to pH variations from 4.2 to 3.0, as a function of the added volume of HCl. Carbonate system parameters (*p*CO<sub>2</sub>, CO<sub>3</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup> and DIC concentrations, saturation states of calcite and aragonite) are calculated from pH (in total scale), total alkalinity, temperature and salinity using a program by Lewis & Wallace.

### Biological parameters

On each field visit, extensive SCUBA surveys are made to photograph and sample macroorganisms present within and adjacent to vent areas to build a voucher collection of the species present and record their distributions. Abundances of macroorganisms are quantified in gridded 0.25 m<sup>2</sup> quadrats placed at 5

m intervals along transects. Point counts are used to determine macroalgal cover and each quadrat is censused fully for macrofaunal abundances, taking specimens for laboratory identification when necessary. Larger organisms, such as sea urchins are counted each 30 m<sup>2</sup> (10 m length x 3 m depth) throughout 200 m long (north shore) and 300 m long (south shore) vent areas. Transplant and colonization experiments are now underway and will be the subject of future publications.

## Results

Vents off Castello Aragonese acidify seawater through gas emissions comprising 90.1-95.3% CO<sub>2</sub>, 3.2-6.6% N<sub>2</sub>, 0.6-0.8% O<sub>2</sub> 0.08-0.1% Ar and 0.2-0.8% CH<sub>4</sub> (no sulphur). Salinity (38‰) and total alkalinity (2.5 mEq kg<sup>-1</sup>) are homogenous between survey stations and temperature matches ambient seasonal fluctuations (13-27°C). On the southern side of the island gas is emitted at 1.4 x10<sup>6</sup> l day<sup>-1</sup> in an area of about 3,000 m<sup>2</sup> (mainly >5 vents m<sup>-2</sup>); on the north side gas is emitted at 0.7 x10<sup>6</sup> l day<sup>-1</sup> in an area of about 2,000 m<sup>2</sup> (mainly <5 vents m<sup>-2</sup>). No seasonal, tidal or diurnal variation in gas flow rates was detected in 2006-2009. The pH of calcite and aragonite vary with sea state, being lowest on calm days, and exhibited large decreases as pCO<sub>2</sub> levels increased from ca 300 to > 2000 µatm through the venting gas fields. Tables 1-2 present the macroalgal and macrofaunal species recorded along 300 m stretches of shore on north and south sides of Castello Aragonese in spring 2007.

**Table 1.** Macroalgal presence (dots) along 35 m sections of rocky shore in spring 2007 at mean pH 8.14 (stations N<sub>1</sub>, S<sub>1</sub>), 7.87 (N<sub>2</sub>), 7.83 (S<sub>2</sub>), 7.09 (N<sub>3</sub>) and 6.57 (S<sub>3</sub>) adjacent to CO<sub>2</sub> vents off Ischia, Italy. Calcifiers are shown in bold, note reduced numbers of species at N<sub>2</sub>, S<sub>2</sub> and their absence at N<sub>3</sub> and S<sub>3</sub>. Total numbers of taxa at each station are given at the end of the table.

Taxa	N1	S1	N2	S2	N3	S3	Taxa	N1	S1	N2	S2	N3	S3
Phaeophyceae	<i>Acerabularia acetabulum</i>		*				<i>Codium effusum</i>	*	*	*	*		
	<i>Bryopsis plumose</i>	*	*				<i>Codium vermilara</i>	*		*	*		
	<i>Caulerpa prolifera</i>	*	*	*	*		<i>Udotea petiolata</i>	*	*	*	*		
	<i>Caulerpa racemosa</i>	*	*	*	*	*	<i>Halimeda tuna</i>	*	*				
	<i>Chaetomorpha</i> sp.	*	*	*	*	*	<i>Pedobesia simplex</i>	*	*				
	<i>Cladophora prolifera</i>	*	*	*	*		<i>Rhizoclonium tortuosum</i>	*	*	*	*		*
	<i>Cladophora rupestris</i>	*	*	*	*	*	<i>Valonia utricularis</i>	*	*	*	*		
	<i>Codium bursa</i>	*	*	*	*		<b>Other Corallinaceae</b>	*	*	*	*		
	Rhodophyceae	<i>Acrosorium ciliolatum</i>	*	*				<i>Gulsonia nodulosa</i>	*	*	*	*	
<i>Aglaothamnion tenuissimum</i>		*	*				<i>Hildenbrandia rubra</i>	*	*	*	*	*	*
<i>Amphiroa rigida</i>		*	*				<i>Jania longifurca</i>	*	*				
<i>Pterothamnion plumula</i>		*	*	*	*		<i>Jania rubens</i>	*	*				
<i>Apoglossum ruscifolium</i>		*	*	*	*		<i>Laurencia obtusa</i>	*	*	*	*	*	*
<i>Asparagopsis taxiformis</i>		*	*	*	*	*	<i>Lithophyllum incrustans</i>	*	*				
<i>Ceramium</i> sp.		*	*	*	*		<b><i>Mesophyllum lichenoides</i></b>	*	*				
<i>Ceramium virgatum</i>		*	*	*	*		<i>Peyssonnelia squamata</i>	*	*	*	*		
<i>Chondracanthus feedei</i>		*	*	*	*	*	<i>Polysiphonia cf. stricta</i>	*	*		*		
<i>Corallina elongata</i>		*	*										
<i>Corallina officinalis</i>		*	*										
Phaeophyceae	<i>Cladostephus spongiosus</i>	*	*	*	*	*	<i>Halopteris filicina</i>	*	*	*	*	*	*
	<i>Colpomenia sinuosa</i>	*	*	*	*	*	<i>Kuckuckia cf. spinosa</i>	*	*				
	<i>Cutleria</i> sp.	*	*	*	*	*	<i>Padina pavonica</i>	*	*				
	<i>Cystoseira amentacea</i>	*	*	*	*		<i>Ralfsia verrucosa</i>	*	*	*	*	*	*
	<i>Cystoseira compressa</i>	*	*	*	*		<i>Sargassum vulgare</i>						
	<i>Diclyoptera polydoides</i>	*	*				<i>Sphacelaria citrosa</i>	*	*	*	*	*	*
	<i>Diclyota dichotoma</i>	*	*	*	*	*	<i>Sphacelaria fusca</i>	*	*	*	*	*	*
	<i>Diclyota fasciola</i>	*	*				<i>Stypocaulon scoparium</i>	*	*	*	*	*	*
	<i>Diclyota spiralis</i>	*	*	*	*	*	<i>Turf</i>	*	*	*	*	*	*
<b>Total number of taxa</b>	<b>49</b>	<b>51</b>	<b>34</b>	<b>36</b>	<b>15</b>	<b>20</b>							

**Table 2.** Macrofauna recorded (dots) along 35 m sections of rocky shore in spring 2007 at mean pH 8.14 (stations N1, N2, N3, S1, S2, S3), with external shells or skeletons shown in bold. Total numbers of taxa at each station are given at the end of the table, note reduced numbers of species at N2 and S2 and further reductions at N3 and S3.

Taxa		N1	S1	N2	S2	N3	S3	Taxa	N1	S1	N2	S2	N3	S3
Sponges	<i>Ageias oroides</i>		*					<i>Dysidea</i> sp.		*		*	*	*
	<i>Cacospongia</i> sp.	*	*	*	*	*		<i>Haliclona mediterranea</i>	*	*	*	*		
	<i>Chondrilla nucula</i>	*	*	*	*	*		<i>Ircinia variabilis</i>	*	*	*	*		
	<i>Chondrosia reniformis</i>	*	*	*	*	*		<i>Spirastrella cunctatrix</i>	*	*	*	*	*	*
Cnidarians	<i>Actinia equina</i>	*	*	*	*	*	*	<i>Balanophyllia europaea</i>	*	*				
	<i>Aglaophenia pluma</i>	*	*	*	*	*	*	<i>Caryophyllia smithii</i>	*	*				
	<i>Anemonia viridis</i>		*					<i>Cladocora caespitosa</i>	*	*				
	<i>Eudendrium</i> sp.	*	*	*	*	*	*	<i>Parazoanthus axinellae</i>	*	*			*	
Amni- liids	<i>Pomatoroceros iniqueter</i>	*	*	*				<i>Serpulidae</i>	*	*	*	*		
	<i>Sabella spallanzanii</i>		*		*			<i>Spirorbidae</i>	*	*	*	*		
Crusta- ceans	<i>Balanus perforatus</i>	*	*	*				<i>Pachygrapsus marmoratus</i>	*	*	*	*	*	*
	<i>Chthamalus stellatus</i>	*	*	*	*	*	*	<i>Palaemon serratus</i>	*	*		*		*
	<i>Euraphia depressa</i>	*	*	*	*	*								
Mollusca	<i>Acanthochitona crinita</i>	*	*	*				<i>Lima lima</i>	*	*				
	<i>Aplysia depilans</i>		*		*	*	*	<i>Melaraphe neritoides</i>	*	*	*	*	*	*
	<i>Arca noae</i>	*	*	*				<i>Mytilus galloprovincialis</i>	*	*	*	*	*	*
	<i>Astrea rugosa</i>	*						<i>Ocenebrina edwardsi</i>	*	*		*		
	<i>Anomia ephippium</i>	*	*	*	*	*	*	<i>Ostraea</i> sp.	*	*				
	<i>Bryum reticulatum</i>	*	*	*	*	*	*	<i>Ostlinus turbinata</i>	*	*	*	*	*	*
	<i>Buccinulum corneum</i>	*	*	*				<i>Patella caerulea</i>	*	*	*	*	*	*
	<i>Cerithium vulgatum</i>	*	*	*	*	*	*	<i>Patella rustica</i>	*	*	*	*	*	*
	<i>Elysia timida</i>	*	*	*	*	*	*	<i>Patella ulyssiponensis</i>	*	*	*	*	*	*
	<i>Flabellina</i> sp.	*	*	*	*	*	*	<i>Serpulorbis arenarius</i>	*	*				
	<i>Fasciolaria lignaria</i>	*	*	*	*	*	*	<i>Thais haemastoma</i>	*	*	*	*	*	*
	<i>Jujubinus striatus</i>	*	*	*	*	*	*	<i>Trochila pullus</i>	*	*	*	*	*	*
	<i>Hydrobia</i> sp.	*	*	*	*	*	*	<i>Octopus vulgaris</i>	*	*	*	*	*	*
	<i>Hexaplex trunculus</i>	*	*	*	*	*	*	<i>Vermetus iniqueter</i>	*	*				
<i>Lepidochitona cinerea</i>	*	*												
Echino- derms	<i>Arbacia lixula</i>	*	*	*	*	*	*	<i>Holothuria tubulosa</i>	*	*	*	*	*	*
	<i>Coscinasterias tenuispina</i>	*	*	*	*	*	*	<i>Holothuria forskali</i>	*	*	*	*	*	*
	<i>Echinaster sepositus</i>	*	*	*	*	*	*	<i>Paracentrotus lividus</i>	*	*	*	*	*	*
Fish	<i>Chromis chromis</i>	*	*	*	*	*	*	<i>Sarpa sarpa</i>	*	*				
	<i>Diplodus annularis</i>	*	*	*	*	*	*	<i>Scorpaena porcus</i>	*	*	*	*	*	*
	<i>Labrus bimaculatus</i>	*	*	*	*	*	*	<i>Symphodus roissali</i>	*	*	*	*	*	*
	<i>Labrus viridis</i>	*	*	*	*	*	*	<i>Trypterion tripteronotus</i>	*	*	*	*	*	*
	<i>Muraena helena</i>	*	*	*	*	*	*							
<b>Total number of taxa</b>									<b>57</b>	<b>69</b>	<b>40</b>	<b>49</b>	<b>15</b>	<b>11</b>

One of the most obvious effects of the CO<sub>2</sub> vent system is the dissolution of shelled organisms and their absence from waters with a mean pH <7.1 (Figure 2).



**Fig. 2** A) General view of *Posidonia oceanica* meadow with CO<sub>2</sub> vents at 2 m depth, May 2009. B) *Patella caerulea* living naturally at mean pH 7.4 showing shell dissolution. C) *Cladocora caespitosa* transplanted to mean pH 7.4 showing skeletal dissolution.

## Discussion

We have shown that natural CO<sub>2</sub> vents can provide insights into which species are tolerant of long-term high CO<sub>2</sub> levels and can be used to test predictions based on modelling and laboratory work, such as what levels of CO<sub>2</sub> exposure restrict the ability of marine organisms to build shells (Fig 2). Although lush stands of seagrasses thrived at increased CO<sub>2</sub> levels (Fig 2), major groups such as corals, sea urchins and bivalves were removed from the ecosystem and replaced by algae such as *Sargassum* sp. and *Caulerpa* spp. (Tables 1&2). In brief, our work shows:

- major ecological tipping points along a gradient of increasing CO<sub>2</sub> levels
- acidification dissolved the shells of calcified species such as corals, sea urchins and snails, which were absent in areas with a pH less than 7.4
- high CO<sub>2</sub> favoured the production of seagrass and removed its calcareous epiphytes
- the amount of calcified algae, which bind coral reefs together in the tropics, fell from more than 60 per cent cover outside the vent areas to zero within these areas
- invasive alien species, which cause damage to ecosystems worldwide, may thrive at high CO<sub>2</sub> levels

Scientific diving work at natural CO<sub>2</sub> vents (Hall-Spencer et al., 2008, Martin et al., 2008, Rodolfo-Metalpa et al. in prep) is providing the first data on what happens to coastal marine ecosystems when key groups of species are killed due to rising CO<sub>2</sub> levels. Having proven our concept with preliminary studies, we recommend that our approach is adopted and supported more widely given the need to gain a sound understanding of the socioeconomic implications of ocean acidification. We are now undergoing the fastest rate of ocean acidification the Earth has seen for at least the past 20 million years so this study adds urgency to the international policy drive to reduce CO<sub>2</sub> emissions.

## Acknowledgements

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# Marine shallow-water hydrothermal systems as natural laboratories

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**Abstract.** Marine shallow-water hydrothermal systems were discovered at more than 40 locations. The discharge of hot mineralized fluids into near shore marine environments creates dramatic physical, chemical and biological gradients. This, combined with easy accessibility, makes them excellent “natural” laboratories to study a wide range of chemical, physical, and biological processes. Studies can be performed in the form of passive thought experiments or actively by inserting experiments into the system. These types of experiments possess the potential to overcome several of the limitations of „normal“ laboratory environments.

## Introduction

Research on seafloor hydrothermal activity has focused primarily on deep-sea black smoker-type locations, which are found along volcanically active portions of the mid-ocean ridges and in deep back-arc basins. Submarine hydrothermal activity, however, is not confined to deepwater environments. Hydrothermal vents have been documented on the tops of seamounts, on the flanks of volcanic islands, and in other near-shore environments characterized by high heat flow (e.g., Dando and Leahy, 1993; McCarthy et al., 2005; Prol-Ledesma et al., 2004). Their easy accessibility, relative to deep-sea hydrothermal systems, makes them excellent “natural” laboratories to study a wide range of chemical, physical, and biological processes.

The purpose of this contribution was not to give an all-encompassing account of the use of marine shallow-water hydrothermal systems as natural laboratories. Rather the intent is to introduce the idea and to demonstrate their utility on hand of select examples. In addition some basic information about the relationship between, geology, water source, heat source and discharge depths are given to aid in the search of marine shallow-water hydrothermal systems with desired physico-chemical conditions.

## Abundance of marine shallow-water hydrothermal systems

Approximately 40-50 sites are presently known. However considering that many are found in remote, poorly explored areas of the globe it is likely that for every known there will be several unknown localities. Their occurrence is closely controlled by geologic and tectonic conditions, because a large heat differential is needed to initiate hydrothermal circulation (Fehn and Cathles, 1986). This heat differential is generally caused by heat released from intruding magma bodies or by the generation of frictional heat along fractures. As a result, most marine shallow-water hydrothermal systems are found near ocean island volcanoes (mantle hot spots), island arc volcanoes (subduction zones) and large active faults (transform faults).

## Characteristics of marine shallow-water hydrothermal systems

Unfortunately there is no clear definition of what marine shallow-water hydrothermal systems are, nor is there any clear agreement how to call these phenomena. This author defines them as: the submarine discharge of a hydrothermal fluid into the shallow ocean (<200 m); they can have characteristics of sub aerial hot springs or that of deep-sea hydrothermal systems; the hydrothermal fluid source can include any fraction of meteoric water; phase separation (boiling) during or immediately prior to discharge is likely; they discharge into the photic zone and may contain appreciable amounts of dissolved oxygen.

The term “marine shallow-  
script and conforms to what is currently used in the scientific literature (Aliani et al., 1998; Ishibashi et al.,