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Monterey Bay cold-seep biota: Assemblages, abundance, and ultrastructure of living foraminifera

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Abstract

Although there is a growing body of evidence indicating benthic foraminifera inhabit hydrocarbon and cold seep environments, biochemical and ultrastructural data on seep foraminiferal communities are not available. Therefore, sediments collected from cold seeps in Monterey Bay, CA (900–1000 m), were examined for the presence of live benthic foraminifera. Results from three independent methods (ATP assay, ultrastructural analysis, rose Bengal staining) indicate that certain species inhabit the Clam Flat and Clam Field seeps. Abundances in our seep samples were lower than in comparable non-seep sites, although not atypical for these bathyal depths. Of 38 species represented at these two seep sites by cytoplasm-containing specimens, only *Spiroplectammina biformis* was restricted to the seep environment. However, because *S. biformis* is also known from non-seep sites in other areas, it should not be considered as endemic to seeps. Ultrastructural studies show abundant peroxisomes in seep specimens, which may allow inhabitation of such environments. One specimen of *Uvigerina peregrina* had prokaryotes nestled in test pores, suggesting that bacteria may play a role in the survival of foraminifera in this seep environment.

Keywords: Foraminifera; Meiobenthos; Protist; USA; California; Monterey Bay

1. Introduction

Because cold seeps are a source of oceanographically and atmospherically relevant compounds such as methane and sulfide, reliable proxies of seep activity are needed to reconstruct the

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temporal patterns of fluid release. Although the species composition of foraminiferal assemblages and the chemistry of their tests (shells) are considered reliable proxies for a variety of paleoceanographic parameters and processes, foraminiferal inhabitation of cold seeps is equivocal. In fact, in a thorough review of cold-seep fauna (Sibuet and Olu, 1998), there is no mention of the foraminifera. Even though hydrogen sulfide and methane are toxic to most eukaryotes once a critical threshold concentration is exceeded, and even though foraminifera are aerobes, certain foraminifera inhabit sulfidic, oxygen-depleted environments (reviewed in Bernhard, 1996; Bernhard and Sen Gupta, 1999) suggesting that foraminiferal inhabitation of seeps is likely. A number of studies have analyzed foraminifera obtained from seeps (Kaminski, 1988; Shirayama and Ohta, 1990; Jones, 1993; Montagna et al., 1989; Akimoto et al., 1994; Sen Gupta and Aharon, 1994; Sen Gupta et al., 1997; Buck and Barry, 1998; Rathburn et al., 2000), but none have employed protocols that unequivocally quantify *living* foraminifera from both seep communities and nearby non-seep sites. For example, previous studies of foraminifera associated with seeps have not discriminated between cytoplasm-containing tests and empty tests (Kaminski, 1988; Shirayama and Ohta, 1990; Jones, 1993) or have used the non-vital stain rose Bengal (Montagna et al., 1989; Akimoto et al., 1994; Sen Gupta and Aharon, 1994; Sen Gupta et al., 1997; Rathburn et al., 2000), so it is uncertain whether the foraminifera were alive at the time of sampling (Bernhard, 1988; 2000; Hannah and Rogerson, 1997; see however Murray and Bowser, 2000). Sen Gupta et al. (1997) examined cytoplasmic ultrastructure in an attempt to show that foraminifera live at seeps, but none of the specimens appeared alive at the time of primary fixation. Bernhard and Bowser (1999) noted that the foraminifer *Buliminella elegantissima* (reported as *Bulimina elegantissima*) collected from a shallow-water seep contained healthy cytoplasm (determined by ultrastructural studies), but quantitative data and a complete community description were not provided.

Here we present results based on two independent vital methods (adenosine triphosphate concentration, cellular ultrastructure) and, for comparative purposes, conventional rose Bengal (rB) staining, to determine if foraminifera inhabit methane- and sulfide-enriched cold seeps in Monterey Bay, off central California, USA.

2. Methods

Two active cold-seep sites and adjacent non-seep areas were targeted for this study (Table 1; Fig. 1). Samples were collected eight times between October 1997 and April 1999 from the Clam Field and the Clam Flat cold seep sites (36°44.0′N, 122°2.6′W and 36°44.7′N, 122°16.6′W; water depths 906 and 1003 m, respectively). Pore fluids emanating from both sites reach high sulfide concentrations (to >5 mM; Barry et al., 1997) but methane concentrations in Clam Flat are much higher than those of Clam Field (311 vs. 11 μM; Barry et al., 1996). Only Clam Field samples had mats of sheathed, filamentous sulfide oxidizing bacteria of the Beggiatoaceae. Because these bacteria and the vesicomyid clams also found at the seeps require hydrogen sulfide and either nitrate or only trace concentrations of oxygen (e.g., Jørgensen and Gallardo, 1999), they are visible indicators of a known chemical environment. Additional information on the chemistry, geology and megafauna of these areas can be found in Barry et al. (1996, 1997). The non-seep site

Table 1
Sample location information

Area	Sample designation	Date	General location/site attributes	Analysis
Clam field	Field 1	13 Oct 97	Amidst clams, bacterial mats	rB, TEM
	Field 2	18 June 98	With bacterial mats	rB, TEM
	Field 3	10 July 98	With bacterial mats	rB, TEM, ATP
	Field 4	8 Dec 98	With bacterial mats	rB, ATP
	Field 5	9 Mar 99	With bacterial mats	ATP
	Field 6	9 Apr 99	With bacterial mats	rB
	Field 7	9 Apr 99	With bacterial mats	rB
	Field 8	13 Apr 99	With bacterial mats	rB, ATP
	Field non-seep	13 Apr 99	>5 m away from seep	rB, ATP
Clam flat	Flat 1	17 Apr 98	Amidst clams	rB, TEM
	Flat 2	17 Apr 98	Edge of clams	rB
	Flat 3	17 Apr 98	Edge of clams	rB
	Flat non-seep 1	17 Apr 98	~ 20 m away from seep	rB
	Flat non-seep 2	17 Apr 98	>50 m away from seep	TEM
	Flat non-seep 3	17 Apr 98	>50 m away from seep	TEM

at Clam Flat was located 20 m from the bacterial mats and clams that define the extent of the seep. The non-seep site at Clam Field was located >5 m away from the seep.

Samples were obtained with the Monterey Bay Aquarium Research Institute's (MBARI) remotely operated vehicle (ROV) *Ventana*. Sediment cores of 7-cm diameter were taken using the manipulator arm in coordination with the onboard camera and, after ROV recovery, transferred to the support ship RV *Pt. Lobos*. Cores were maintained in a cooler on ice during transport to MBARI for subsequent processing, which usually occurred within a few hours of collection. In an environmental room at ambient temperature (6°C), cores were subsampled with 1.3-cm diameter syringe cores, from which the surface centimeter was removed and processed.

Samples intended for determination of foraminiferal adenosine triphosphate concentration ([ATP]) were maintained at ambient temperature or on ice during specimen isolation. Sediment aliquots were sieved briefly with chilled 0.2 μm-filtered seawater (FSW) over a 75-μm screen. Specimens from the >75-μm fraction were isolated, rinsed in FSW and extracted individually for ATP following the procedure of Bernhard (1989). Luciferase activity was assayed using a EG&G Berthold LUMAT 9501 luminometer. Data were analyzed according to the procedure of Bernhard and Reimers (1991), which adopted a higher live–dead threshold than that used by Bernhard (1992).

Samples intended for rB enumeration, transmission electron microscopy (TEM), or scanning electron microscopy (SEM) were fixed in chilled 2% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2). Because these subsamples were also used for studies of nano-, micro- and other meiofauna, sample splits were used for most analyses. For rB counts, aliquots were stained with a saturated solution of the lipophilic stain for ~ 24 h, sieved over a 63-μm screen with distilled

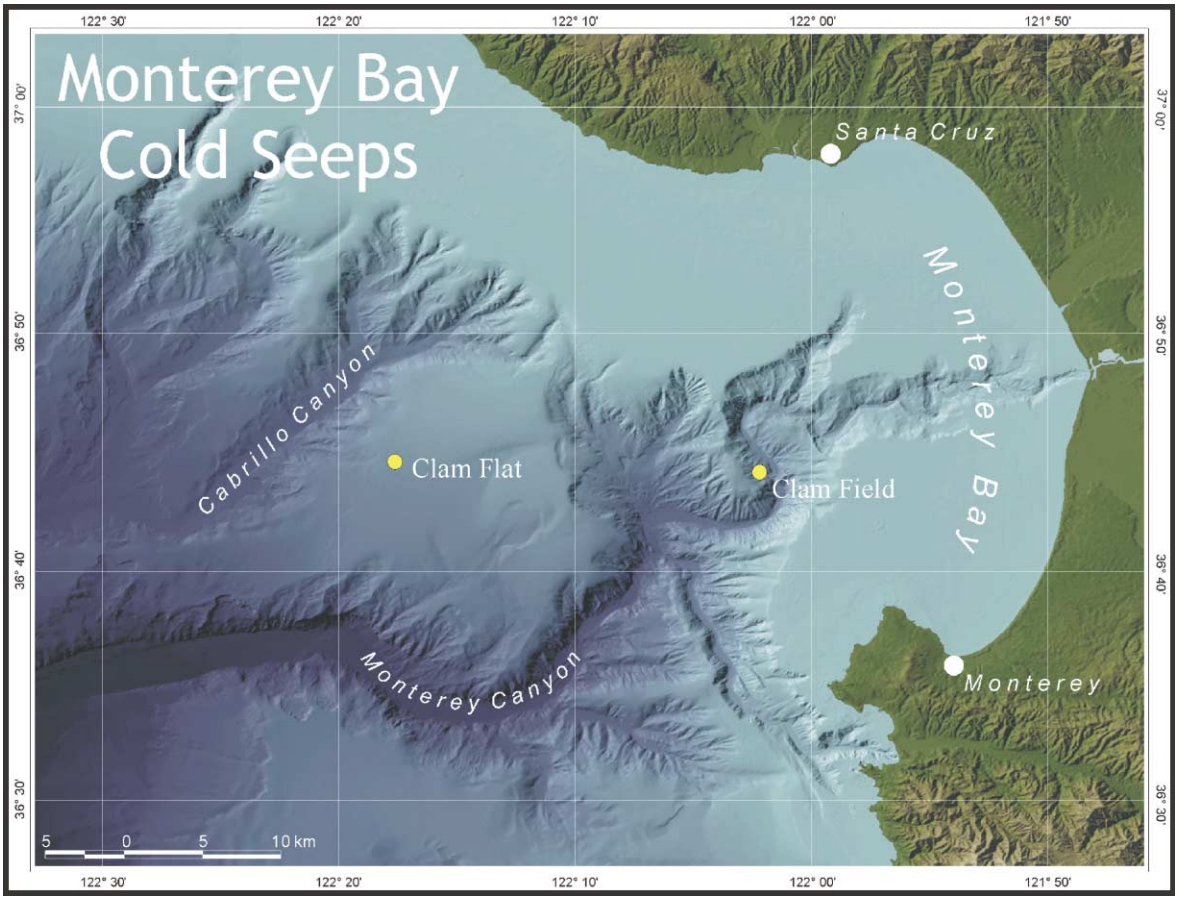

Fig. 1. Map showing the location of Clam Flat and Clam Field seep sites.

water, and the coarser fraction examined using a Nikon stereoscope. All foraminifera staining bright pink in more than one chamber were identified and enumerated from each available subsample or split. Rarefaction plots were calculated using BioDiversity Pro, which is available at http://www.nrmc.demon.co.uk/bdpro.

Specimens for ultrastructural observation were obtained from sample splits that were incubated briefly ($\sim$2 h) with rB before specimen isolation and subsequent processing. The brief rB incubation facilitated isolation of cytoplasm-filled specimens. After sediments were sieved with chilled FSW over a 63-μm screen, stained specimens were isolated from the $>$63-μm fraction and processed for TEM using standard methods (Bernhard and Alve, 1996). Sections were examined with a JEOL 100B. Specimens intended for documentation with SEM were removed from sieved sediments, immersed in $\sim$5% hypochlorite for 5–10 min to remove organic debris, rinsed thoroughly in distilled water, and air-dried. After specimens were mounted on stubs and sputter coated with Au + Pd, they were examined with an ISI ABT or Hitachi S-2500Δ.

3. Results

3.1. ATP

Because adenosine triphosphate is always present in live cells, we used it as an unequivocal indicator of benthic foraminifera inhabiting Monterey Bay cold seeps. Living *Bolivina pacifica*?, *Buliminella tenuata*, *Epistominella pacifica*, *Fursenkoina rotundata*, *Loxostomum pseudobeyrichi*, "*Textularia*" sp., *Tolypammina* sp., and *Uvigerina peregrina* were present in Clam Field bacterial mat samples (Table 2). For three samples assayed for both rB and ATP (i.e., Field 4, 8, Field Non-Seep), specimens deemed living by ATP assay belonged to species that were also typically stained in the corresponding rB split (see below). However, although *Globobulimina* spp. constituted the dominant rB-stained taxa in the Field 3 sample, none of the *Globobulimina* extracted for ATP from that sample were living at the time of extraction ($n = 14$). Specimens from Clam Flat were not assayed for ATP.

3.2. Ultrastructure

Typical foraminiferal organelles appeared intact in numerous specimens ($n = 12$) from many seep samples (Fig. 2). Mitochondria, peroxisomes, and Golgi apparatus were observed in a variety of species (Table 3; Fig. 2A). Peroxisomes were commonly complexed with endoplasmic reticulum (Fig. 2B), as observed in other foraminifera from oxygen depleted, sulfidic environments (Nyholm and Nyholm, 1975; Bernhard and Alve, 1996; Bernhard, 1996). Evidence of ectobionts was present in only one *Uvigerina peregrina* from Clam Flat. The specimen harbored prokaryotes in its pores, typically just above the pore plug (Fig. 2C). All bacteria observed ($n \sim 20$) were similarly rod shaped.

Table 2
ATP results[a]

Sample	Field 3[b]	Field 4	Field 5	Field 8	Field non-seep
n	53	42	36	63	70
Bolivina pacifica?	0/1	1/2	0/0	0/0	0/0
Buliminella tenuata	6[c]/9	3/3	1/3	4/4	21/34
Epistominella pacifica	2/16	1/11	1/22	15/41	0/8
Fursenkoina rotundata	0/0	3/10	0/1	0/0	0/0
Loxostomum pseudobeyrichi	0/0	0/0	0/0	1/1	0/0
"*Textularia*" sp.	0/0	1/1	0/0	0/0	1/1
Tolypammina sp.	0/0	0/0	0/0	1/2	0/0
Uvigerina peregrina	0/3	1/5	0/4	1/4	0/7

[a] n = number extracted per sample. Fractions reflect number of individuals of a given species determined to be live at the time of extraction vs. number of that species extracted in that sample (i.e., 2/5 indicates two of five were alive using ATP criteria). Denominators will not sum to the total because other species, for which none were alive, were also extracted.

[b] This was a quantitative sample; resultant live foraminiferal density = 2.8/cm^2.

[c] Includes one specimen that was an empty test (i.e., contamination occurred, most likely from bacteria).

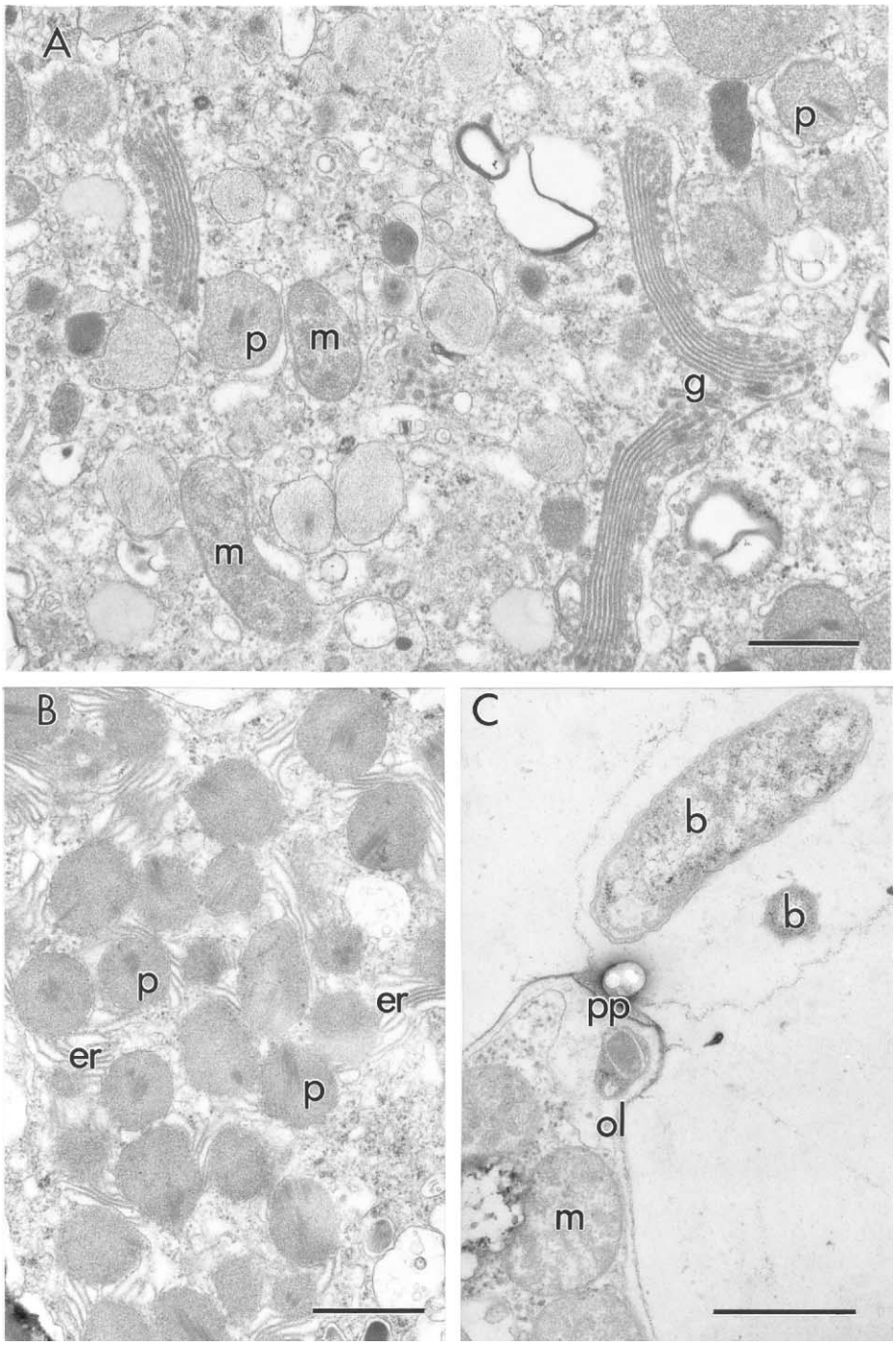

Fig. 2. Transmission electron micrographs of benthic foraminifera from Monterey Bay cold seep samples. (A) *Praeglobobulimina spinescens*, Clam Field. Note the paired Golgi apparatus (g) and individual peroxisomes (p). (B) *Buliminella tenuata*, Clam Field. Note the complex of peroxisomes with endoplasmic reticulum (er). (C) *Uvigerina peregrina*, Clam Flat. Note the prokaryotes (b) nestled in pore above pore plug (pp). The calcareous test would have been outside the organic lining (ol). Also note the mitochondria (m) clustered beneath the pore plug. Scale bars = 1 μm.

Table 3
Synopsis of ultrastructural observations[a]

Species	*n*	Sample	Organelles observed
Bolivina spissa	1	Flat 3	M, P
Buliminella tenuata	3	Flat 3	M
		Field 1	M, P, ER
		Field 2	M, P, ER, G
Epistominella pacifica	1	Field 1	M
Globobulimina sp.	2	Field 1	M, P, ER
		Field 3	no intact organelles (i.e., dead)
Loxostomum pseudobeyrichi	1	Flat 3	M, P
Praeglobobulimina spinescens	2	Flat 1	M, P, G
		Field 3	M, P, G
Uvigerina peregrina	2	Flat 2	M, P, G; prokaryotes in pores
		Field 1	M, P

[a] *n* = number examined with TEM. Although omitted from table, at least one specimen per species was examined from non-seep sites. M = mitochondria, P = peroxisomes, ER = endoplasmic reticulum, G = Golgi apparatus. Nuclei were not observed, probably because specimens were shallowly sectioned (i.e., not deep into the cell where nuclei typically occur).

3.3. Rose Bengal

Even though the rB method has limitations, we present rB data for two reasons: (1) to allow comparison to seep studies that have used only this stain to distinguish "live" specimens, (2) to show general foraminiferal distribution patterns and community attributes. It is imperative to remember that a specimen brightly stained with rB merely indicates the presence of cytoplasm, but nothing about the viability of that cytoplasm (see Bernhard, 2000, for discussion). Because samples were not replicated and specimen abundance was low in some samples, we refrained from statistical analyses; patterns should be viewed with appropriate caution. Rare species and those occurring in less than four samples were not considered.

Abundances of cytoplasm-containing foraminifera were substantially lower in samples from Clam Field than in nearby non-seep samples or any samples from Clam Flat (Table 4). Because our samples widely ranged in the number of recovered individuals, rarefaction is considered appropriate in such cases for comparisons of species richness (see however, Lambshead et al., 2000). Rarefaction plots indicate that species richness of cytoplasm-containing foraminiferal tests (i.e., expected number of species) was lower at Clam Field than Clam Flat (Fig. 3), although it should be noted that 4 of 12 samples had particularly low specimen yields (16–21 specimens) that could affect diversity estimates. Table 4 also suggests species richness is lower at Clam Field compared to Clam Flat. In addition, rarefaction plots indicate species richness in seep samples is similar to comparable non-seep samples (Fig. 3).

The following species were generally more abundant and typically made up higher proportions in seep samples compared to non-seep samples: *Cassidulina delicata*, *Epistominella pacifica*, and *Spiroplectammina biformis* (Fig. 4; Table 4). *Epistominella pacifica* occurred, often in high abundances (i.e., 36.2 cm^{-2}), in all but one seep sample, was absent in the Clam Field non-seep sample, and represented by only 2 specimens in the Clam Field non-seep sample. *Epistominella*

Table 4
Rose Bengal stained foraminifera from the 0–1 cm interval of Monterey Bay cold seep and non-seep samples[a]

	Clam field								Clam flat				Average (#/cm^2)		Average (%)	
	Field 1	Field 2	Field 3	Field 4[b]	Field 6	Field 7	Field 8[b]	Non-seep[b]	Flat 1	Flat 2	Flat 3	Non-seep	Seep	Non-seep	Seep	Non-seep
Adelosina sp.	0.7												0.1(0.2)	0	0.7(2.1)	0
Ammodiscus incertus			0.7								1.4	0.7	0.2(0.5)	0.4(0.5)	0.8(1.9)	0.4(0.5)
Astrononion sp.	0.7												0.1(0.2)	0	0.7(2.1)	0
Bolivina pacifica?	0.7	0.7		2.3	1.5		1.3	2.3	2.1		0.7	0.7	0.8(0.7)	1.5(1.1)	4.1(3.6)	2.3(2.3)
Bolivina spissa (?)				0.6	0.4			0.4	9.7	9.7	5.6	21.6	2.8(4.3)	11.0(15.0)	4.2(6.2)	10.9(14.5)
Bolivina sp.												0.7	0	0.4(0.5)	0	0.4(0.5)
Bulimina mexicana	0.7			0.6	0.2				1.4	2.8	0.7	3.5	0.6(0.9)	1.8(2.5)	1.5(2.1)	1.7(2.4)
Buliminella tenuata	1.4	0.7	1.4	8.8	1.3	0.2	0.6	11.1	0.7	0.7	9.7	9.7	1.9(3.0)	10.4(1.0)	6.6(4.5)	14.35(6.9)
Cassidulina delicata		0.7			0.4				4.9	2.8	2.1	1.4	2.1(1.7)	0.7(1.)	2.4(3.0)	0.7(1.0)
Chilostomella oolina					0.2			2.3		1.4	0.7		0.3(0.5)	1.2(1.6)	0.4(0.6)	9.6(13.6)
Cibicides sp.											0.7		0.1(0.2)	0	0.1(0.3)	0
Epistominella exigua	0.7			1.2		0.2		0.2	2.1	2.1		2.8	0.6(0.9)	1.5(1.8)	1.9(2.5)	1.5(1.7)
Epistominella pacifica	2.1	7.6	2.1	9.4	8.5	1.5	6.1	0.9		36.2	20.9		9.4(11.8)	0.5(0.6)	32.3(18.6)	0.8(1.1)
Epistominella smithi									14.6	4.2	2.1	7	2.3(4.8)	3.5(4.9)	3.7(8.4)	3.4(4.8)
Fontbotia wuellerstorfi									0.7	1.4	0.7		0.3(0.5)	0	0.4(0.6)	0
Fursenkoina rotundata				50.3	1.1	0.8	2.3	0.6		1.4	0.7		0.7(0.8)	0.3(0.4)	5.0(7.9)	0.5(0.7)
Fursenkoina cornuta										0.7			0.1(0.2)	0	0.1(0.3)	0
Globobulimina spp.	1.4		5.6	6.4	1.7	0.4	0.2	0.6				0.7	1.0(1.8)	0.7(0.1)	8.4(14.3)	0.9(0.2)
Gyroidina altiformis									5.6	3.5	0.7	1.4	1.1(2.0)	0.7(1.0)	1.6(3.3)	0.7(1.0)
Lagenosolenia sp.											0.7		0.1(0.2)	0	0.1(0.3)	0
Loxostomum pseudobeyrichi							0.2		0.7	1.4		4.9	0.3(0.5)	2.5(3.5)	0.5(0.7)	2.4(3.4)
Miliammina sp.												0.7	0	0.4(0.5)	0	0.4(0.5)
Nonion sp.								0.2					0	0.1(0.1)	0	0.2(0.2)
Nonionella globosa?			0.7					2.8					0.1(0.2)	1.4(2.0)	0.6(1.9)	2.5(3.5)
Phthanotrochus arcanus		0.7	0.7	1.8			0.4	21.5	2.1	2.8	8.3	14.6	1.7(2.7)	18.1(4.9)	3.5(3.5)	25.7(16.1)

Praeglobobulimina spinescens								1.3	1.4	0.7	0.7	1.4	0.3(0.5)	1.4(0.1)	0.5(0.8)	1.7(0.4)
Psammosphera sp.				0.6							1.4		0.2(0.5)	0	0.2(0.6)	0
Reophax gracilis												0.7	0	0.4(0.5)	0	0.4(0.5)
Reophax sp. 1			0.7	2.3	0.2	0.2	0.2	0.6					0.1(0.2)	0.3(0.4)	1.4(2.2)	0.5(0.7)
Reophax sp. 2				0.6				0.2				0.7	0	0.5(0.4)	0	0.5(0.3)
Spiroplectammina biformis							0.2		2.1	4.2	0.7		0.8(1.5)	0	1.2(1.8)	0
"*Textularia*" sp.	1.4	1.4		6.4	0.9	0.2	0.4	11.5		2.8	13.2	7.6	2.3(4.2)	9.6(2.8)	8.8(6.5)	13.7(8.7)
Tolypammina sp.							0.2						0.0(0.1)	0	0.2(0.5)	0
Trifarina angulosa									0.7				0.1(0.2)	0	0.1(0.4)	0
Triloculinella sp.						0.2							0.0(0.1)	0	0.5(1.6)	0
Trochammina sp.		0.7						0.2					0.1(0.2)	0.1(0.1)	0.6(1.9)	0.2(0.2)
Uvigerina peregrina	0.7			2.3	2.1	0.4	0.6	0.6	7	9	6.3	20.2	2.9(3.5)	10.4(13.9)	6.7(4.4)	10.4(13.2)
Uvigerina sp.												0.7	0	0.4(0.5)	0	0.4(0.5)
Verneuilinula? sp.									1.4				0.2(0.5)	0	0.3(0.8)	0
allogromid					0.2								0.0(0.1)	0	0.1(0.3)	0
saccamminid				5.3	1.7			0.2					0.2(0.6)	0.1(0.1)	0.9(2.7)	0.2(0.2)
planispiral agglutinate	0.7		0.7		0.9			0.6			0.7	0.7	0.3(0.4)	0.7(0.1)	1.9(2.7)	0.9(0.2)
juvenile calcareous				1.2				0.4					0	0.2(0.3)	0	0.4(0.5)
Total # picked	16	18	18	171	113	21	66	56.2	82	126	113	147	—	—	—	—
#/cm^2	11.1	12.5	12.5	NA	21.3	4	12.5	58.1	57	87.6	78.6	102.2	33.0(32.3)	80.2(31.2)	—	—
# species	11	7	8	16	15	9	11	20	16	18	21	21	—	—	—	—

[a] Species data per sample represent abundance (#/cm^2). Data for Field 4 is non-quantitative, so italicized values represent proportion of the rB assemblage (%) rather than abundance. Four columns on the right are pooled abundance and percentage data from all seep samples and both non-seep samples. Parenthetic values are standard deviations. Also given is total number of rB stained specimens picked and total abundance (#/cm^2) for each sample. Blank entries indicate lack of stained specimens.

[b] Includes specimens determined to be live by ATP assay.

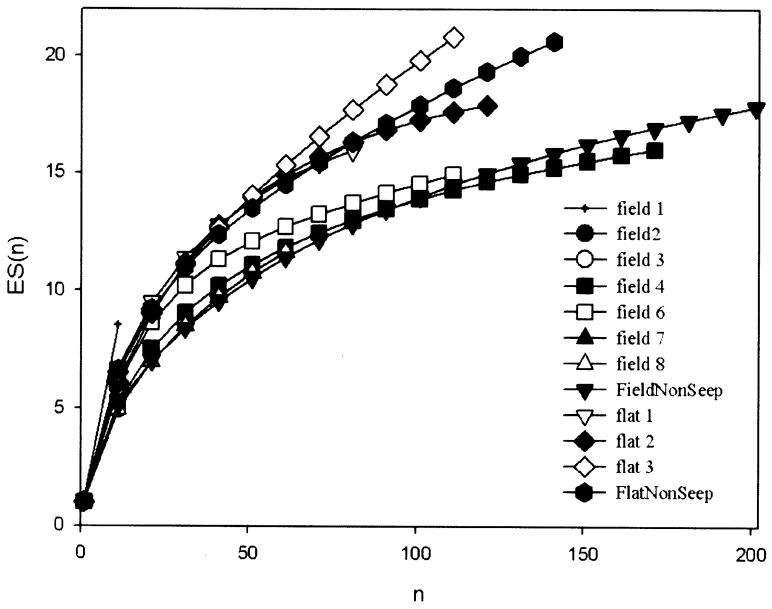

Fig. 3. Rarefaction curves based on rose Bengal stained foraminiferal counts from individual samples. ES(n) = Expected number of species; n = number of specimens.

smithi, *Fursenkoina rotundata*, and *Gyroidina altiformis* had higher densities in one or two seep samples and generally made up a larger proportion of the stained assemblage in seep samples compared to non-seep samples. *Globobulimina* spp. had higher proportions in Clam Field seep samples compared to the corresponding non-seep sample. Species that generally occurred in similar proportions in both seep and non-seep samples were *Bolivina pacifica*, *Bolivina spissa*, *Bulimina mexicana*, *Epistominella exigua*, and *Praeglobobulimina spinescens*. In contrast, the proportion and abundance of *Phthanotrochus arcanus* were lower in cores from seeps than from nearby non-seep samples; "*Textularia*" sp. exhibited roughly the same pattern. *Uvigerina peregrina* occurred in all but two samples, with high abundance in all Clam Flat samples (seep and non-seep). *Buliminella tenuata* occurred in all samples, with higher abundances in both non-seep samples and some seep samples.

The abundances of species common at seeps differed among seep sites. *Epistominella smithi* and *Gyroidina altiformis* were found exclusively in Clam Flat samples while *Globobulimina* spp. were limited to Clam Field samples, except for a single specimen in the Clam Flat non-seep sample. *Bolivina spissa*, *Bulimina mexicana*, *Cassidulina delicata*, *Praeglobobulimina spinescens*, and *Spiroplectammina biformis* occurred in all Clam Flat seep samples. Only *Reophax* sp. 1 was present more consistently in Clam Field than Clam Flat samples.

4. Discussion

Because the mitochondrion is the first organelle to exhibit visible changes during cell death (reviewed in Wyllie, 1987), the presence of mitochondria with cristae in our specimens confirms

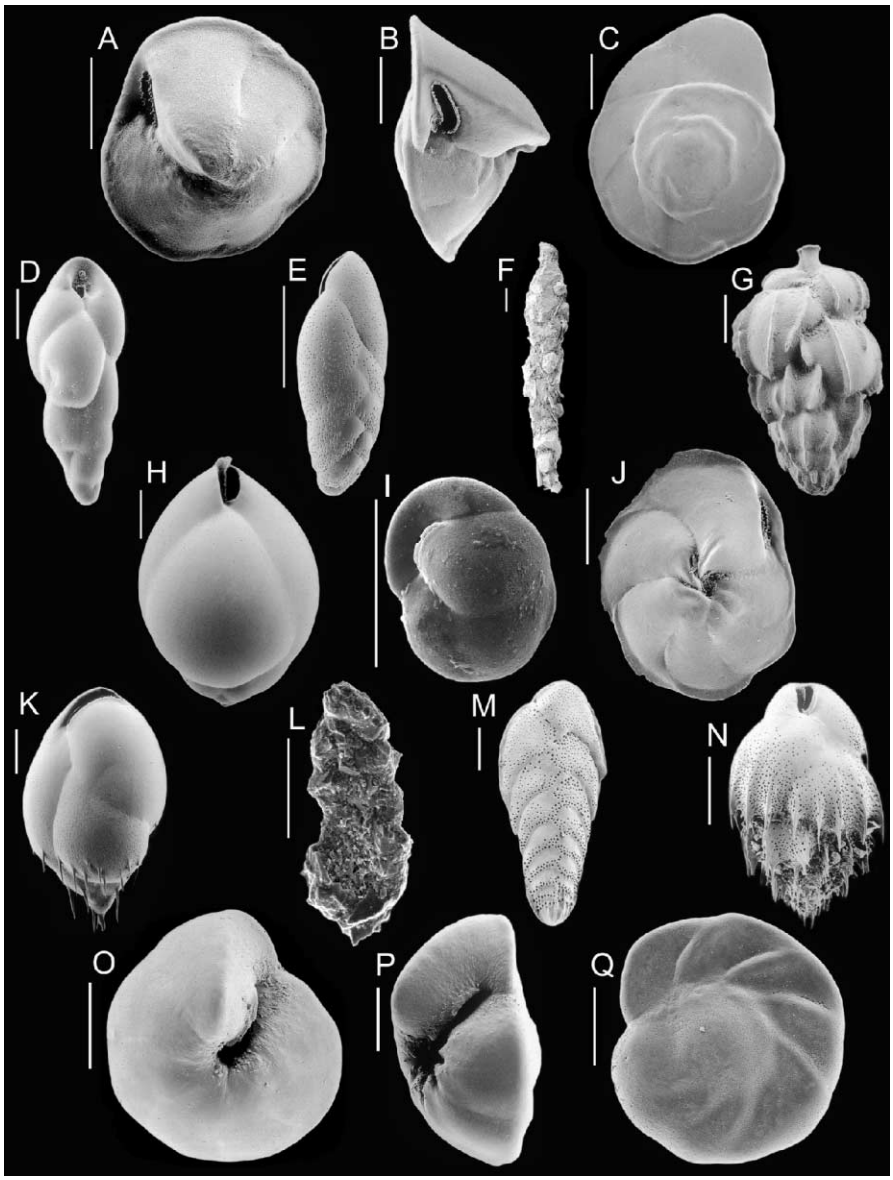

Fig. 4. Scanning electron micrographs of representative Monterey Bay seep foraminiferal species. (A, B, C) *Epistominella pacifica*, (D) *Buliminella tenuata*, (E) *Fursenkoina rotundata*, (F) *Reophax* sp. 1, (G) *Uvigerina peregrina*, (H) *Globobulimina* sp., (I) *Cassidulina delicata*, (J) *Epistominella smithi*, (K) *Praeglobobulimina spinescens*, (L) *Spiroplectammina biformis*, (M) *Bolivina spissa*, (N) *Bulimina mexicana*, (O, P, Q) *Gyroidina altiformis*. Scale bars = 100 μm.

that foraminifera inhabit both Clam Flat and Clam Field seeps. Independent ATP data supports the conclusion that foraminifera inhabit cold seeps in Monterey Bay, CA. It is not clear if the foraminifera inhabiting Monterey Bay seep habitats have specific physiological adaptations to

seep environments or if they inhabit microhabitats lacking sulfide and methane, thereby negating their need for detoxification mechanisms.

4.1. Physiological adaptations

A considerable number of mega- and macrofaunal taxa inhabiting seeps have symbionts (~30%; Sibuet and Olu, 1998), and four of six common foraminiferal species from a Beggiatoaceae mat in the Santa Barbara Basin have symbionts (Bernhard et al., 2000). Therefore, one might expect to find symbionts associated with Monterey Bay seep foraminifera. Ultrastructural studies did not reveal, however, the presence of any obvious cellular adaptations (e.g., endosymbionts) to allow foraminifera to inhabit these sulfidic environments. The prokaryotes associated with pore plugs in *Uvigerina peregrina* could be considered ectosymbionts. Although we have no information concerning the ectobiont's physiology, these prokaryotes may enable their foraminiferal hosts to inhabit these environmental conditions. To our knowledge, prokaryotic ectobionts on foraminifera have not been described previously. *Buliminella tenuata* from Monterey Bay seeps did not have endobionts, in contrast to conspecifics from the Santa Barbara Basin (Bernhard, 1996; Bernhard et al., 2000). Because specimens from Monterey Bay lack endobionts, we conclude that this association between protist and prokaryote is not an obligatory symbiosis. This plastic relationship could provide insights into symbiogenesis.

Another possible ultrastructural adaptation of foraminifera to the seep environment is the presence of numerous peroxisomes, which are commonly complexed with endoplasmic reticulum. Peroxisomes are the site of glycolysis, an anaerobic metabolic pathway (e.g., Masters and Crane, 1995). Although all healthy specimens are expected to have peroxisomes, their proliferation may benefit specimens inhabiting seeps and may be necessary for their survival. On the other hand, because peroxisomes are the site of many other cellular metabolic processes and metabolism regulation (Masters and Crane, 1995), it is possible that additional pathways are responsible for foraminiferal survival in seep environments.

The cellular organization of species associated exclusively with seeps (i.e., only *Spiroplectammina biformis* in our samples) may provide clues concerning the survival of these aerobic protists in such potentially toxic environments. Ultrastructural studies of this and other species that are common in seep environments (e.g., *Cassidulina delicata*, *Epistominella smithi*, *Fursenkoina rotundata*, *Gydroidina altiformis*) will help resolve this cell-biological enigma.

4.2. Microhabitats

Because we do not know the actual life position of the foraminifera in the upper cm, it is possible that the foraminifera examined in this study were living in microhabitats with environmental conditions more typical of the deep sea and unlike those of cold seeps. Although the chemistry of pore fluids in Monterey Bay seeps varies considerably both between and within seeps (Barry et al., 1997), the high abundance of euglenozoa with epibiotic sulfur-oxidizing bacteria found in some of these samples (Buck et al., 2000) indicates that sulfide is present in the top cm. It is possible, however, that the levels of sulfide are kept below the critical threshold of toxicity to foraminifera by the activities of these epibiotic sulfide-oxidizing prokaryotes. Other seep inhabitants, including both vesicomyid clams with sulfide-oxidizing endosymbionts and filamentous, free-living sulfide-

oxidizing bacteria (*Thioploca* spp.), are also capable of localized depletion of sulfide concentrations. Only fine-scale geochemical and ecological sampling will resolve this question.

It is also possible that the foraminiferal cell body and, therefore, test (i.e., shell) reside in sulfide- and/or methane-enriched pore waters but their pseudopodia extend into overlying oxygen-laden bottom waters. Because mitochondria are shuttled throughout pseudopodial networks (Doyle, 1935; Travis and Bowser, 1991; J.M. Bernhard, unpubl. obs.), oxidative phosphorylation may occur in a site remote from the main cytoplasmic body, but at the resolution of classical benthic ecology, foraminifera inhabit the Monterey Bay cold seeps.

4.3. Foraminiferal community attributes

Cold seeps in Monterey Bay are sites of enhanced biomass for bacteria (Barry et al., 1996; McHatton et al., 1996), vesicomyid clams (Barry et al., 1996, 1997), nematodes (Buck and Barry, 1998), and some protists (Buck and Barry, 1998). However, this does not appear to be the case for foraminifera. Our data suggests foraminiferal abundance in Monterey Bay is lower at seeps than at non-seep sites. This conclusion is supported by the observation that foraminiferal biovolume was lower at the Clam Field seep site compared to non-seep sites (Buck and Barry, 1998). Foraminiferal diversity at Monterey Bay seeps appears comparable to nearby non-seep sites, although this conclusion should be considered with appropriate caution because of the limitations of our data. Rose Bengal-estimated densities for our seep samples fall within the range for similar bathyal depths in the eastern Pacific (e.g., Mackensen and Douglas, 1989; Bernhard, 1992). Species diversity of foraminifera in Monterey Bay seeps may, however, be lower than found in other non-seep habitats from comparable depths (Gooday, 1999). Studies that included other meiofauna, such as nematodes (Buck and Barry, 1998), also noted fundamental differences between the characteristics of the cold seep fauna and those at shallower (50 m) chemoautotrophic bacterial mat-dominated areas (Jensen, 1987; Bernard and Fenchel, 1995).

4.4. Species and generic trends

Our results support previous observations of rB-stained foraminiferal communities at cold seeps that indicate species composition is not consistent between seeps and that few species, if any, are endemic to seeps. Indeed, our rB data add to the growing list of seep-associated species, and suggest that much remains to be learned about these habitats. Foraminiferal assemblages reported from seep environments are probably influenced greatly by fluid chemistry near the sediment surface. Foraminiferal communities (as determined by rB) from hydrocarbon-dominated seeps (e.g., Sen Gupta and Aharon, 1994; Sen Gupta et al., 1997) differ considerably from those in Monterey Bay, possibly because Monterey Bay surface sediments and fluid discharge are influenced less strongly by hydrocarbons than in the Gulf of Mexico. Regarding endemism, although *Uvigerina peregrina* is reported from seeps in several studies (Jones, 1993; Sen Gupta and Aharon, 1994; Sen Gupta et al., 1997; Rathburn et al., 2000, this study), it was not observed in Sagami Bay seeps (Akimoto et al., 1994). Furthermore, *U. peregrina* also occurred abundantly in Monterey Bay non-seep samples and, thus, should not be considered as a "diagnostic" seep indicator, as suggested by Kohn et al. (1998). A number of other species that occur in both Monterey Bay seeps and seeps off northern California (i.e., *Bolivina spissa*, *B. pacifica*, *Buliminella*

tenuata, *Chilostomella oolina*, *Epistominella smithi*, *Loxostomum pseudobeyrichi*, *Nonionella globosa*; this study, Rathburn et al., 2000) are also common in non-seep settings along the California margin (e.g., Culver and Buzas, 1986; Bernhard et al., 1997).

Some consistencies are observed, however, at the generic level among rB seep data sets from widely separated seeps. For instance, *Bulimina* species are reported in most seep data sets (*B. striata*, Sagami Bay, Western Pacific, Akimoto et al., 1994; *B. alazanensis*, Gulf of Mexico, Sen Gupta et al., 1997; *B. mexicana*, CA margin, Rathburn et al., 2000; this paper). *Cassidulina* species are also often observed in seep samples (*C. laevigata*; North Sea, Jones, 1993; *C. carinata* and *C. norvangi*, Akimoto et al., 1994; *C. neocarinata*, Sen Gupta and Aharon, 1994; Sen Gupta et al., 1997; *C. curvata* and *C. subglobosa*, Sen Gupta and Aharon, 1994; *C. delicata*, this study). It is relevant to note, however, that both genera can be common in non-seep habitats (Murray, 1991).

Although no species can be considered a seep endemic, some are as common or more common in seep samples than non-seep samples. For example, the abundance of *Rutherfordoides cornuta* and *Bulimina striata* is enhanced in seeps (Akimoto et al., 1994). In this study, *Cassidulina delicata*, *Epistominella pacifica*, *Fursenkoina rotundata*, and *Spiroplectammina biformis* were typically more common in seep samples than non-seep samples. Given the patchiness of foraminiferal distributions in the deep sea (Bernstein et al., 1978), this conclusion should also be viewed with caution due to the poor replication in non-seep samples.

4.5. Comparison to Santa Barbara Basin foraminiferal community

Besides hydrocarbon seeps and hydrothermal vents, mats of sulfide-oxidizing Beggiatoaceae bacteria occur at bathyal depths in the silled Santa Barbara Basin (SBB) off southern California (Soutar and Crill, 1977; Bernhard et al., 2000). Although the Monterey Bay seeps are deeper than SBB (900–1000 m vs. $\sim$600 m), there are several environmental similarities between these areas. Both lie within the oxygen minimum zone and have a flux of hydrogen sulfide across the sediment–water interface (Barry et al., 1997; Kuwabara et al., 1999).

The foraminiferal fauna from SBB has been studied extensively (e.g., Phleger and Soutar, 1973; Bernhard and Reimers, 1991; Bernhard et al., 1997; Bernhard and Bowser, 1999; Cannariato et al., 1999). Foraminiferal densities in SBB are considerably higher than observed in Monterey Bay seeps. For example, foraminiferal populations in SBB (determined by ATP analysis) can be $>700\,cm^{-3}$ in the top 0.25 cm (Bernhard and Reimers, 1991), while comparable estimates of foraminiferal density in Monterey Bay seeps were two orders of magnitude lower ($2.8\,cm^{-3}$, top cm; Table 2). A similar trend occurs in the rB data, with densities in SBB to $>1000\,cm^{-3}$ (top 0.25 cm; Bernhard and Reimers, 1991), compared to Monterey Bay seep samples where abundance was always $<100\,cm^{-3}$ (top cm). Although many foraminifera in the SBB and Monterey Bay seeps are congeners, the same species do not commonly occur in both areas. Only *Buliminella tenuata* occurs consistently in SBB and Monterey Bay seeps. The maximum density of *B. tenuata* in SBB was $53\,cm^{-3}$ (Bernhard et al., 1997), but its maximum density was $<10\,cm^{-3}$ in Monterey Bay seeps (Table 4). This species occurs in even lower numbers at seeps off northern California (e.g., $<1\,cm^{-3}$; Rathburn et al., 2000).

It is likely that differences in SBB and Monterey Bay foraminiferal assemblages result from the variations in spatial and temporal attributes of the two areas. Although the pore-water chemistries are similar at both locales, the environmental chemistry of SBB appears to be stable

over both space and time compared to that found at Monterey Bay seeps. The SBB below sill depth is >20 km in diameter while Monterey Bay seeps are typically <3 m in diameter (Barry et al., 1996, 1997). Oxygen-depleted conditions in the SBB have persisted, with occasional brief aeration events, for about the last ten thousand years (Cannariato et al., 1999), while cold seeps probably persist for much shorter periods and may manifest considerable variability in the fluid chemistry of the upper sediment column. The variability in pore fluid chemistry, lateral extent, and short longevity of cold seeps may explain the faunal differences between seep sites as well as the faunal differences between the seeps and other sulfide-enriched environments.

4.6. Conclusions

Two independent methods indicate unequivocally that benthic foraminifera inhabit two cold seeps in Monterey Bay. Abundance and diversity appear to be quite low, but within the range of typical bathyal values. No foraminiferal 'indicator species' were identified, however, from these two seep locations. Unexpectedly (and with the exception of one *Uvigerina peregrina* individual), seep specimens did not harbor prokaryotic symbionts, which potentially could facilitate foraminiferal inhabitation of such environments. Even though no endemic or diagnostic seep species were identified, it is possible to glean useful proxy data from foraminiferal species that reside both in seeps and around them (Sen Gupta and Aharon, 1994; Kennett et al., 2000; Rathburn et al., 2000). For example, estimates of seep activity and intensity might be obtained by comparing stable-isotope data from seep specimens to that of non-seep conspecifics (Rathburn et al., 2000). Still lacking, however, are detailed studies of micro-scale gradients in pore fluid chemistry coupled with foraminiferal studies employing appropriate viability assays. Such studies would help resolve the role of environmental controls and biogeochemistry on faunal patterns in these habitats that are presumed stressful to aerobic eukaryotes such as foraminifera.

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