

Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow

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ABSTRACT: Relationships among fluid flow, sulfide concentration, sulfur bacteria and macrofaunal assemblages were examined at methane seeps on the northern California margin, near the mouth of the Eel River (512 to 525 m). Over a 6 mo period, sediments covered with microbial mats exhibited significant but variable outflow of altered fluids, with no flow reversals. This fluid flow was associated with high porewater sulfide concentrations (up to 20 mM) and almost no oxygen penetration of sediments (<0.1 mm). *Vesicomya pacifica* (clam) bed and non-seep sediments exhibited little net fluid outflow and similar oxygen penetration (3 and 4 mm, respectively); however, sulfide concentrations were higher in subsurface clam-bed sediments (up to 2 mM) than in non-seep sediments (<200 µM). Macrofaunal densities did not differ among the 3 habitats (13 800 to 16 800 ind. m⁻²; >300 µm), but biomass and diversity (no. species per core, E(S₁₀₀), H') were lower and composition varied in the sulfidic microbial mat sediments relative to clam-bed and non-seep sediments. The community in microbial mat-covered sediments consisted largely (82%) of 6 species in the polychaete family Dorvilleidae, whereas the clam-bed and non-seep microhabitats supported a mixture of annelids, peracarid crustaceans, nemerteans, and mollusks. Vertical microprofiling of sulfide in animal cores indicated that most taxa avoid H₂S concentrations >1 mM. However, sulfide-oxidizing filamentous bacteria, dorvilleid polychaetes and bivalves (mainly *V. pacifica*) exhibited highest densities at sulfide concentrations of 1 to 5 mM sulfide. Horizontal and vertical patterns of sulfide availability have a strong influence on the fine-scale distribution, structure and composition of macrofaunal assemblages inhabiting methane seeps and must be accounted for when characterizing the microbiology and ecology of seep habitats.

KEY WORDS: Sulfur bacteria · Cold seep · Diversity · Dorvilleidae · Eel River · Macrobenthos · Microbial mat · Sulfide tolerance

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INTRODUCTION

Marine sediments in nearshore and deeper continental margin settings characteristically exhibit a range of redox conditions, including zones of aerobic respiration, sulfate reduction and, in highly organic-

rich sediments, methanogenesis (Aller 1982). Some of the most extreme conditions are found in areas where methane-laden fluids seep upwards, fueling syntrophic microbial reactions involving methane oxidation and sulfate reduction. These interactions yield exceptionally high porewater sulfide concentrations at

methane seeps (Boetius et al. 2000, Valentine 2002). Because sulfide is toxic to most animals (Griesshaber & Volkel 1998), we expect the community structure and distribution of infaunal assemblages in seep sediments to differ from those in surrounding non-sulfidic, non-seep sediments, and to be strongly influenced by sulfide availability (Bernhard et al. 2001, Sahling et al. 2002).

Methane seeps are broadly distributed along the tectonically active eastern Pacific margin (Kulm et al. 1986, Kennicut et al. 1989, Suess et al. 1989, 1998, Barry et al. 1996, Yun et al. 1999). Most research on the fauna of seeps has focused on the large megafauna (clams, tube worms, and mussels) (Sibuet & Olu 1998, Van Dover et al. 2003), or on the microbiota (Buck & Barry 1998, Hinrichs et al. 2000, Orphan et al. 2000). Investigators have described highly specialized, symbiont-bearing megafauna and complex microbial assemblages. Spatial variability in the abundance and composition of selected seep megafauna has been attributed to the influence of methane or sulfide (Barry et al. 1997, Wallman et al. 1997, Freytag et al. 2001). Responses of macro-infauna to seepage have only recently been explored (Sahling et al. 2002).

Infaunal macrobenthic and meiobenthic organisms can be abundant in seep sediments, but little is known about controls on their composition and distribution in these environments (Buck & Barry 1998). The magnitude of fluid flow in seep environments is the primary control on the chemical profile with depth, which in turn is related to the distribution of fauna (Barry et al. 1997, Sahling et al. 2002). Low net flow rates of ~ 1 cm yr^{-1} appear to provide sufficient methane from depth to fuel the near-surface communities while still allowing downward transport and mixing of oxygen- and sulfate-rich seawater in the upper few centimeters of sediment. More intense fluid flow is associated with altered porewater composition and elevated sulfide concentrations that extend to the sediment surface, allowing the growth of surface microbial mats (Tryon & Brown 2001, Torres et al. 2002).

Recently, Sahling et al. (2002) described macrofaunal communities (>500 μm) associated with different seep habitats at Hydrate Ridge on the Oregon margin. They found differences in assemblage density, composition and diversity associated with *Acharax*, *Calyptogena* (*Vesicomya*) and bacterial-mat-dominated sediments that were strongly linked to gradients in sulfide flux and concentration. In a comparison of shallow-water seep and non-seep macrofaunal assemblages (>300 μm) on the northern California shelf (35 to 55 m), Levin et al. (2000) found only minor differences, with several species preferring or avoiding seeps, but no specialists. Preliminary analyses of infauna from 520 m in a *C. (V.) pacifica* bed revealed an assemblage char-

acteristic of non-seep, California margin environments (Levin et al. 2000). However, this initial study focused on only 1 seep habitat—clam beds. It did not examine the community in relation to nearby non-seep sediments or bacterial-mat-covered sediments, and no geochemical data were collected.

Here we present a detailed characterization of seep macrofaunal community structure in relation to fluid flow and sediment porewater geochemistry for several different habitats. We ask: (1) Do sulfide concentrations, the abundance of large, filamentous sulfur bacteria, and infaunal community organization vary consistently among microbial mat, clam-bed and non-seep sediments? (2) Are these differences associated with patterns of fluid flow? (3) Are there macrofaunal taxa specifically associated with distinct seep environments? (4) Do microbial and animal distributions vary vertically within seep sediments in relation to local (millimeter-scale) porewater sulfide concentrations? We hypothesized that patterns of fluid flow create and regulate the geochemical environment, which in turn regulates community composition and dictates the horizontal and vertical distributions of macrofaunal organisms at methane seeps.

MATERIALS AND METHODS

Sediment sampling. Investigations were carried out in a region of extensive seepage ($\sim 500 \times 300$ m) located at 515 to 525 m on the northern California continental slope ($40^\circ 47.08' \text{N}$, $124^\circ 35.68' \text{W}$), offshore of the Eel River mouth. The site contained surficial microbial mats (Fig. 1A) and numerous patches of *Vesicomya pacifica* aggregations (Fig. 1B). The microbial mat habitat was often associated with release of methane bubbles and the occurrence of carbonate rocks. The general region is subject to episodic inputs of flood debris from land (Alexander & Simoneau 1999, Leithold & Hope 1999, Wheatcroft 2002) and sediments contain plant and wood-chip fragments (L. Levin unpubl. obs.). We made 3 CTD casts from the RV 'Thompson' during mid-October 2000 over the study area to measure bottom-water salinity, temperature and oxygen. Bottom sampling was conducted using the remotely operated vehicle (ROV) 'Jason' and control vehicle 'Medea', aboard the RV 'Thompson' from October 12 to 17, 2000. Samples of sediments, fauna and porewaters were collected in 8.3 cm diameter tube cores (30 cm long) from habitats termed 'microbial mat', 'clam bed' and 'non-seep'. Non-seep areas were defined as sediments with no surface manifestation of seep fauna, bacteria or bubbles. Faunal samples included 5 microbial mat, 6 clam-bed and 4 non-seep cores (Fig. 2). All of the microbial mat cores were taken

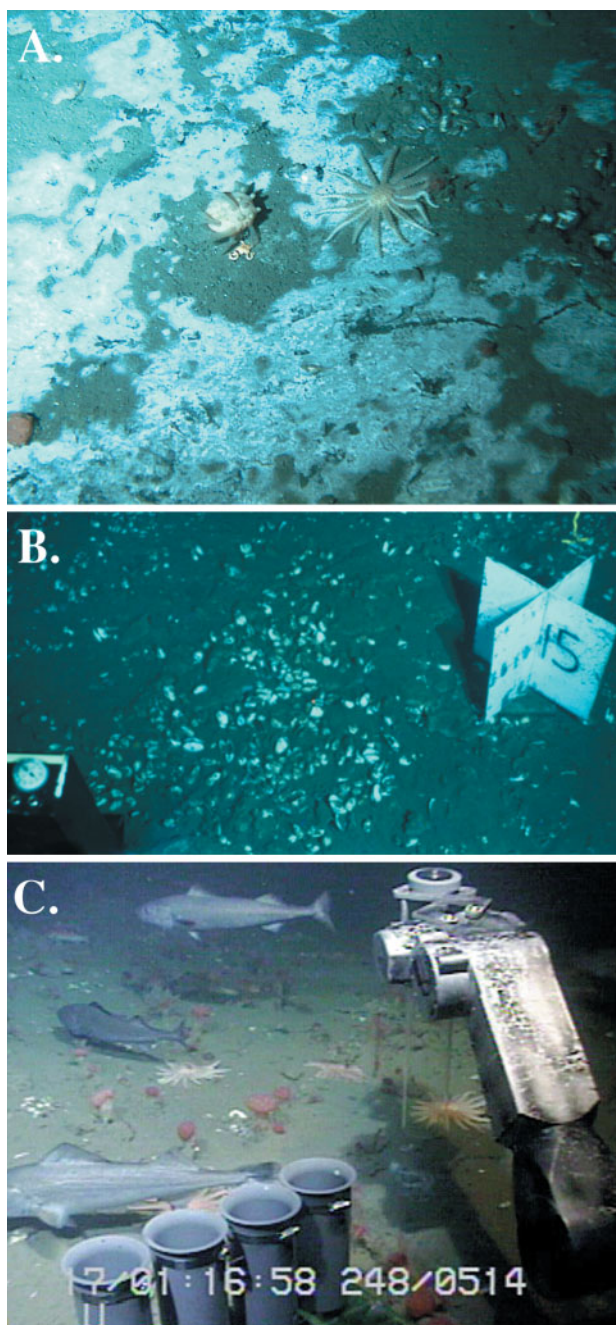


Fig. 1. Eel River margin, Northern California, 525 m. (A) Microbial mat-covered sediments; (B) *Vesicomya* (*Calyptragenia*) *pacifica* aggregations; (C) non-seep sediments

from the southern part of the study area, and thus represent less spatial coverage than the clam-bed and non-seep samples (Fig. 2). Core penetration varied with sediment characteristics, and often was limited to 10 cm depth. Once cores were on board ship, microprofiling of oxygen, sulfide and pH was carried out immediately. The same cores were subsequently pro-

cessed for macrofauna. Cores were stored at 5 to 6°C (ambient bottom-water temperature) until processing.

Sample processing and measurements. Oxygen, sulfide and pH microprofiling: Oxygen and H₂S microgradients were measured in intact cores immediately after retrieval by means of amperometric microelectrodes. This was done prior to sectioning these same cores for quantification of bacteria and macrobenthos.

Vertical oxygen distributions in intact cores were measured by Clark-type microelectrodes provided with a built-in reference and a guard cathode (Jørgensen & Revsbech 1989, Revsbech 1989). The electrodes were purchased from UNISENSE, Denmark, and had a sensing tip of 20 to 40 μm, a stirring sensitivity of <2% and a 90% response time ≤1s. Electrode currents had a linear response to 0 and 100% air-saturation of O₂. Linear calibration was done at 6°C in 100% saturated seawater (35‰) and nitrogen-purged seawater with 0% oxygen-saturation.

H₂S microgradients were measured using miniaturized amperometric sensors with an internal reference and a guard anode (Jeroschewsky et al. 1996). The sensors had a tip diameter of 40 to 60 μm. Calibration was performed after the sensor signal had stabilized during pre-polarization. A stock solution of S²⁻ (100 mM) was prepared by dissolving Na₂S in N₂-flushed 0.1 M NaOH in a closed container. The final concentration of stock solution was determined by standard analysis (Cline 1969). For the calibration curve, suitable amounts of the stock solution were injected into sealed serum vials containing oxygen-free calibration buffer (100 mM phosphate buffer, pH 7). The sensors were immersed in 3 to 4 different calibration solutions. These H₂S sensors were purchased from UNISENSE, Denmark, and have been successfully applied in marine ecological studies (Kuehl et al. 1998).

Oxygen and H₂S electrodes were attached to a micro-manipulator mounted on a heavy stand. The sensors were connected to a high-sensitivity picoammeter (Unisense PA 2000); signals were amplified and data were recorded on a strip chart recorder or directly on a computer. Measurements were typically performed in vertical increments of 250 μm for oxygen and 1 mm for H₂S.

Parallel to the H₂S microprofiles, pH values were measured (5 mm vertical intervals) in the same cores, using a long needle combination pH electrode (Diamond General). The H₂S sensors detect the partial pressure of H₂S gas, which is only one component of the total sulfide equilibrium system. Thus, it is necessary to know the pH of the sample/calibration solution to calculate the total sulfide concentration (Jeroschewsky et al. 1996). We subjected 3 non-seep cores, 6 clam-bed cores and 4 microbial mat cores to microprofile measurements prior to sectioning for macrofauna.

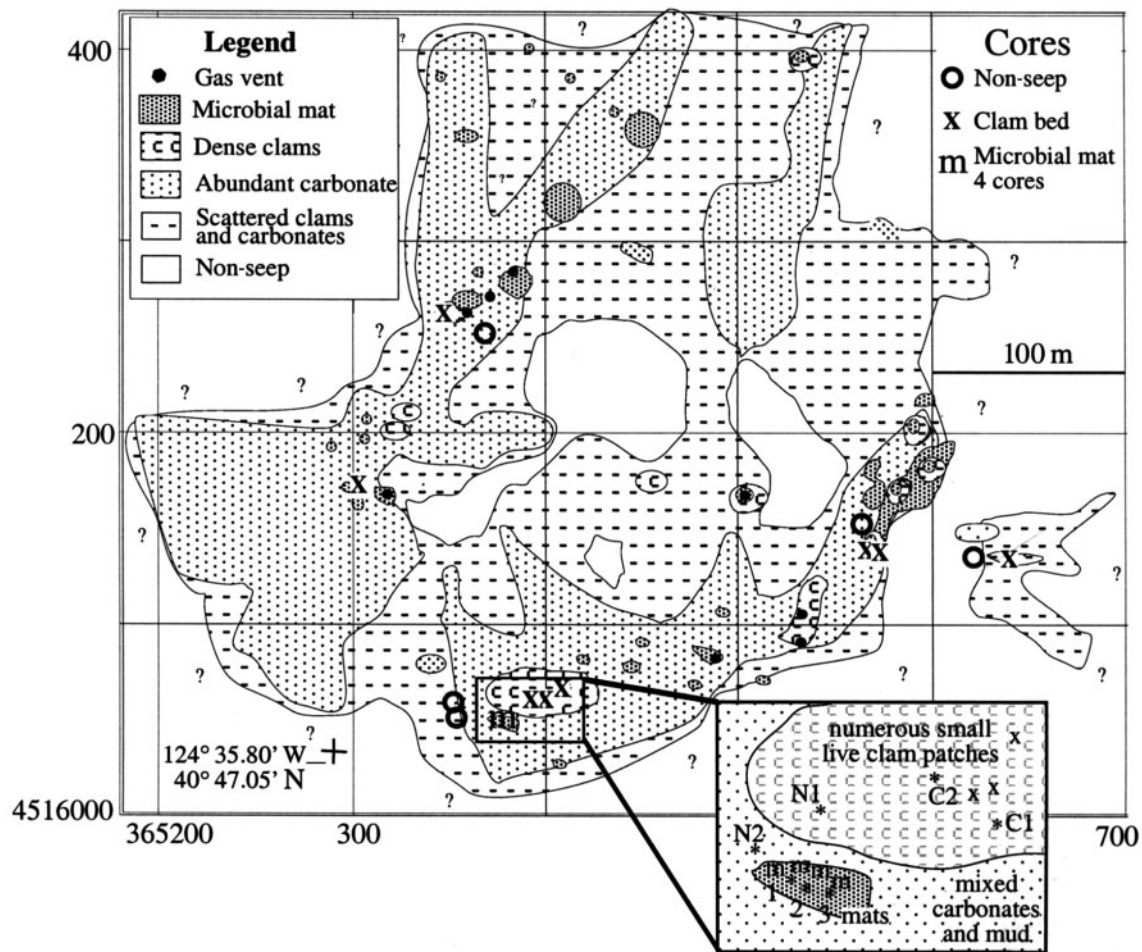


Fig. 2. Core locations and geological and biological features in the study area, Eel River margin, northern California, 500 to 525 m. In inset, * = Flux-meter positions; 1, 2 and 3 = microbial mat sediments; C 1 and C 2 = over clam beds, N1 and N2 = over non-seep sediments

Macrofauna and filamentous bacteria: Most cores to be analyzed for macrofauna and large sulfur bacteria were sectioned vertically at 0–1, 1–2, 2–5, and 5–10 cm intervals. The fractions above 5 cm were preserved unsieved in 8% buffered formaldehyde, to permit analysis of macrofaunal and meiofaunal size taxa. The 5–10 cm fraction was sieved on a 300 μ m mesh prior to preservation. Several additional cores were sectioned at 0.5 cm intervals (to 2 cm) and at 1-cm intervals from 2 to 10 cm, to provide higher resolution information about animal–sulfide relationships. These were preserved without sieving. Oxygen and sulfide profiles were generated for each of these high-resolution cores.

In the laboratory, all previously preserved macrofaunal core sediments were washed in freshwater on a 300 μ m mesh, and retained invertebrates and filamentous bacteria were sorted under a dissecting microscope at 12 \times magnification. Macrofaunal invertebrates were counted and identified to the lowest possible tax-

onomic level (usually species) with the aid of a compound microscope. Bacterial filaments (>300 μ m) were sorted and counted. Sieving may have fragmented these large bacteria, so counts are considered to reflect relative vertical and horizontal patterns but biomass may provide a better indicator of absolute abundance. Sorted invertebrates and bacterial filaments were weighed on an analytical balance to determine wet weight biomass prior to transfer to 70% ethanol.

Habitat differences in macrofaunal density, biomass, taxonomic composition, species richness and other measures of diversity (Shannon information index H' [log-base 2], Pielou's evenness index J' , and Rank 1 dominance, [R1D, as % of most abundant species]) (Magurran 1988) were evaluated. All data were tested for normality with a Shapiro-Wilkes test. Habitat differences were analyzed for normally distributed data with a 1-way ANOVA and, if significant, with an *a posteriori* Tukey's honestly significantly different (HSD) test. Data were analyzed using non-

parametric tests (Kruskal-Wallis with 1-way chi-square approximation) if they were not normally distributed, as transformations failed to normalize most data. JMP software (SAS Institute Inc.) was used to perform univariate statistical tests. Differences in representation of individual taxa were examined using a test based on sample size and the arcsine-transformed percentages (Sokal & Rohlf 1969 p. 607). Multivariate analyses of community structure, including multidimensional scaling (MDS), analysis of similarities (ANOSIM) and SIMPER (similarity percentages) tests, were conducted with PRIMER Software (Primer-E Ltd.) (Clarke & Warwick 1994). PRIMER was also used to calculate diversity indices (H' [log base 2], J' , R1D). For both univariate and multivariate tests we decided not to employ corrections to experimentwise alpha for multiple testing since tests represent separate and clearly defined hypotheses (Quinn & Keough 2002, Hurlbert & Lombardi 2003). Rarefaction curves were generated with Biodiversity Pro (McAleece et al. 1997). Values given for biotic parameters are means \pm 1 SE unless indicated otherwise. Significance is assessed at $\alpha = 0.05$.

Fluid-flow measurements. Measurements of fluid expulsion were made from October 14, 2000 to April 28, 2001, utilizing 7 chemical and aqueous transport (CAT) meters (Tryon et al. 2001). We placed 3 meters with the ROV on microbial mats, 2 in clam beds, and 2 in non-seep sediments (Fig. 2 inset). The meters are designed to quantify both inflow and outflow rates on the order of 0.01 to 1500 cm yr⁻¹. The CAT meter uses the dilution of a chemical tracer to measure flow through the outlet tubing at the top of the chamber. A tracer solution of similar density but different composition from the seep fluid is injected at a constant rate by 2 osmotic pumps into the water stream as it moves through the outlet tubing. These same pumps withdraw a sample of the seep fluid/tracer mixture (in this case scandium and rubidium) from downstream of the tracer injection port, giving a serial record of the tracer dilution. Upon recovery of the instruments, the coils are subsampled at appropriate intervals, and tracer concentration is analyzed via inductively coupled plasma-optical emission spectrometry (ICP-OES).

RESULTS

Visual site observations

A thorough site survey was accomplished early in the first cruise utilizing video and sonar observations from the ROV 'Jason', correlated with track logs from Jason's transponder array (long baseline navigation) accurate to ± 1 m (Fig. 2). Indications of recent and paleo methane seepage (Hovland 2002) were observed

over 70% of the area surveyed, including abundant and widespread small clam (*Vesicomya pacifica*) aggregations, carbonates, less extensive surficial microbial mats and local venting of free methane (Fig. 2). Prior seep activity was indicated by the preponderance of carbonate pavements and low ridges (less than a few meters high) of more rugged carbonate blocks. Large chemohierms, massive carbonates built up due to bacterial mediated CO₂ production from methane and subsequent precipitation of CaCO₃ (sensu Bohrmann et al. 1998), were not observed.

The majority of the *Vesicomya pacifica* were scattered as ~10 to 50 cm diameter aggregations spaced 1 to 5 m apart. Microbial mat sites were typically much rarer and were often manifested as small (10 to 30 cm) patches developed along cracks in carbonates and less commonly in patchy sedimented sites; 1 large mat (a mosaic of patches several meters in diameter) was the site of fluid-flow measurements and coring. Larger aggregations of *V. pacifica* were also observed locally (3 to 10 m diameter) (Fig. 2).

Gas vents typically were observed to consist of several small, dispersed bubbling sites several meters apart, from which intermittent gas release occurred through cracks or at edges of carbonate blocks. Vent sites were generally confined to regions dominated by carbonates. The gas-vent sites typically did not have large microbial mats directly associated with them, although small microbial mats and scattered clam patches occurred locally. Overall, the hydrologic activity in this region was typified by a diffuse network of many small (centimeter-scale) seeps and vents, with diffuse widespread activity over length scales of 100s of meters.

Fluid flow

CAT meter fluid-flow measurements at microbial mat sites revealed significant outflow of altered fluids, with no flow reversals (Fig. 3). During periods of flow, the average rate was 10 cm yr⁻¹. The microbial mat sites, however, exhibited no flow for the first 2 to 3 mo, followed by highly variable outflow for the remaining 4 mo. The altered fluids expelled at these sites were typical of pore fluids in the microbial mat area (J. Gieske & C. Mahn unpubl. data). No difference was observed between fluid flow at clam bed and non-seep sites. These sites exhibited highly variable flow with rapid flow reversals believed to be tidally generated (Tryon et al. 2002). While these instruments cannot resolve flow rates in an environment of rapid flow-reversals, we can qualitatively determine if there is net upflow, downflow, or no net flow (Tryon & Brown 2001). Overall net flow rates from the clam bed and non-seep habitats were low (<1 cm yr⁻¹).

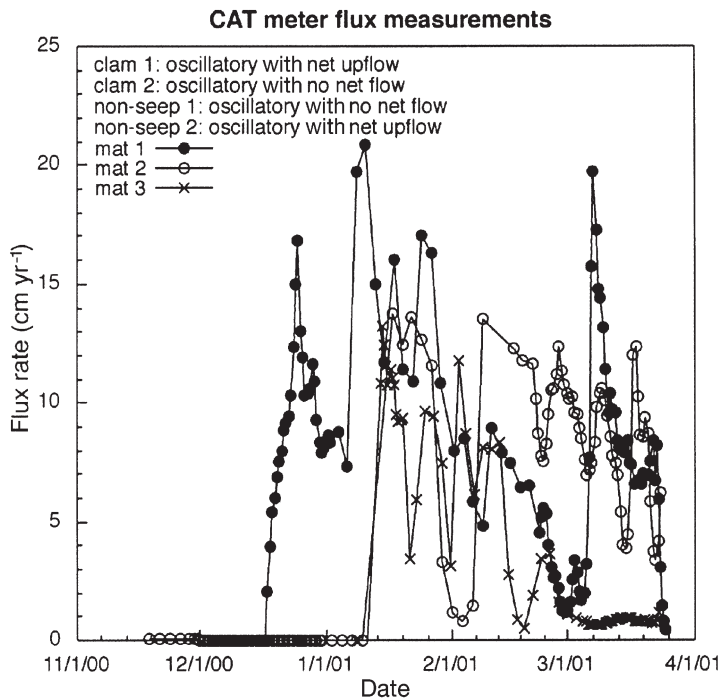


Fig. 3. Fluid-flow determined by CAT (chemical and aqueous transport) fluid flux meters. Dates are given as mo/d/yr. See 'Materials and methods' for instrument description

Sediment porewater geochemistry

The microbial mat, clam bed and non-seep sediments exhibited geochemical and seepage differences in the upper 10 cm of the sediment column. Oxygen penetration was deepest in non-seep sediments (4 mm), but was similar to clam bed sediments (3 mm), with a typical diffusive concentration profile (Jørgensen & Revsbech 1985). In contrast, there was almost no oxygen penetrating microbial mat sediments (<0.1 mM) (Fig. 4).

Each of the habitats studied exhibited a distinctive sulfide profile (Fig. 4). Sulfide concentration was one order of magnitude higher in clambed than non-seep sediments and in microbial mat than clam bed sediments. High concentrations of sulfide (with core maxima of 14 to 20 mM), were measured throughout microbial mat cores and reached the sediment–water interface. The sulfide profiles of the microbial mat core suggest a strong sulfide flux from below (Fig. 4). In the clam

beds, no sulfide was detected in the upper 4 cm, the depth to which we observed the *Vesicomya pacifica* foot to extend in tube cores. Below this zone, sulfide increased with depth to maximum concentrations of up to 2 mM at ~9 cm depth, indicating a well-defined zone of local sulfide production by sulfate reduction (Fig. 4). In non-seep sediments, sulfide was absent or occurred at a low concentration (<0.2 mM) in the upper 3 to 7 cm sediment depth (Fig. 4). The microelectrode data reveal among-microhabitat differences in the amount and distribution of 'sulfide-free' sediment (here defined as <0.1 mM H₂S). This zone was restricted to the surface-water interface in microbial mat sediments, to the upper 4 cm in clam-bed sediments, and spanned the entire sediment column that was sampled (to 12 cm) in most non-seep sediments (Fig. 4).

Overlying water and sediment properties

During the study, bottom-water temperature ranged from 5.28 to 5.75°C, salinity from 33.2 to 33.6, and oxygen concentration from 0.24 to 0.48 ml l⁻¹. Previous analyses of replicate sediment samples collected from clam-bed sediments at this site in 1997 yielded average sediment values of 12.4% CaCO₃, 0.97% organic C, 0.06% organic N, and a C:N ratio of 16.8 (Levin et al. 2000). No additional sediment analyses were conducted in 2000.

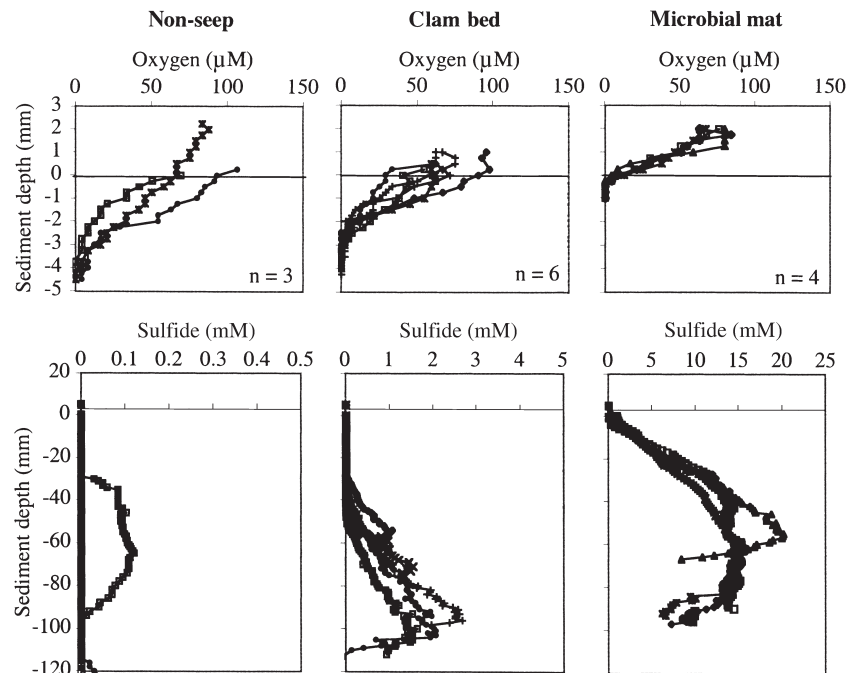


Fig. 4. Microelectrode profiles in microbial mat, clam-bed and non-seep sediments of oxygen and sulfide. Note different scales on various sulfide plots

Megafauna

Most of the study region supported epifaunal communities dominated by cnidarians (*Anthomastus ritteri*), ophiuroids, asteroids (*Rathbunaster californicus*), and large demersal nekton populations comprised largely of sablefish *Anoplopoma fimbria* (Fig. 1). Microbial mats (Fig. 1A) and *Vesicomya pacifica* aggregations (Fig. 1B) were the primary surface manifestation of methane seepage. The vesicomylid clams at Pacific methane seeps form a species complex, but molecular studies confirm the identity of *V. pacifica* in the Eel River region (Goffredi et al. 2002). Microbial mats were comprised largely of *Beggiatoa*-like filamentous bacteria. Shipboard video-feed observations of the sea floor revealed large gastropods *Neptunea* sp. feeding on *V. pacifica*. Sablefish were common in the work area and hovered near the ROV during dives. They were observed stirring up and grabbing mouthfuls of sediment, suggesting that they consume bottom fauna at the seeps.

Filamentous bacteria

Counts of filamentous sulfur bacteria (>300 μm) in each core yielded a consistent pattern: highest values in microbial mat sediments (3428 ± 970 filaments m^{-2}), intermediate values in the clam beds (1442 ± 381 filaments m^{-2}), and lowest values in the non-seep sediments (270 ± 270 filaments m^{-2}). Microbial-mat filament densities (per 10-cm-deep tube core) were higher than those in non-seep sediments, but clam bed densities did not differ from either of the other 2 habitats (Kruskal–Wallis: $p = 0.024$). Total biomass of filamentous bacteria (per 10-cm-deep tube core) exhibited a similar pattern, with highest biomass in the mats (2.25 ± 1.22 g m^{-2}), lowest in non-seep sediments (0.25 ± 0.25 g m^{-2}) and intermediate values in the clam bed (1.80 ± 1.01 g m^{-2}), but differences among habitats were not significant (Kruskal–Wallis: $p = 0.140$).

Macrofaunal community variation in seep habitats

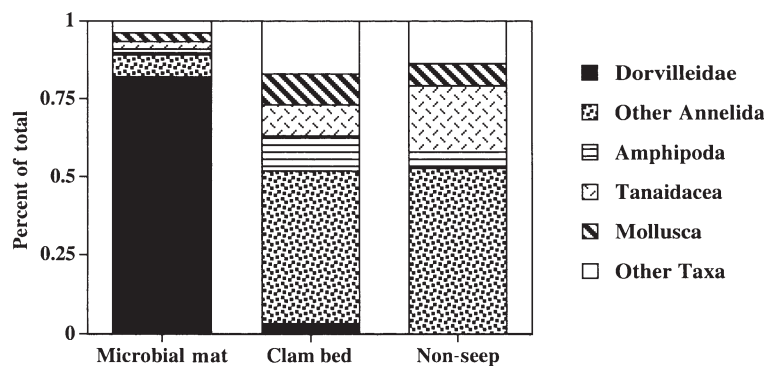
Abundance and composition

Despite strong variation in geochemical conditions, total macrofaunal densities did not vary among seep habitats (1-way

ANOVA: $p = 0.775$). Average densities were 13834 ± 4766 individuals (ind. m^{-2}) in microbial mat sediments, 16892 ± 2277 ind. m^{-2} in clam-bed sediments, and 16552 ± 2112 ind. m^{-2} in non-seep sediments. There was however, significantly less macrofaunal biomass in the mat sediments (1.73 ± 0.40 g m^{-2}), than in clam bed (23.76 ± 11.21 g m^{-2}) or non-seep sediments (23.80 ± 8.53 g m^{-2}) (Kruskal–Wallis: $p = 0.011$).

Strong differences in macrofaunal composition were observed between the microbial mat sediments and the other 2 habitats. Annelids were the dominant phylum in each habitat (Fig. 5A), comprising 89.0, 52.2, and 47.5% of the macrofauna in microbial mat, clam bed, and non-seep sediments, respectively. Microbial mat communities consisted largely of 5 species of dorvilleid polychaetes (82% of the total); the remainder included other annelids (6%), with molluscs, peracarid crustaceans nemerteans, and turbellarians each comprising less than 5% of the fauna (Fig. 5A, Table 1). Composition of the clam bed and non-seep communities was remarkably similar (Fig. 5A), with greater representation of peracarid crustaceans (22.3 and 27.9% [arcsine test for percentage differences: both $p < 0.0001$]), mollusks (9.7% [$p = 0.056$] and 6.3% [$p = 0.124$], respectively), and nemerteans (13.3%

A. Macrofauna



B. Annelida

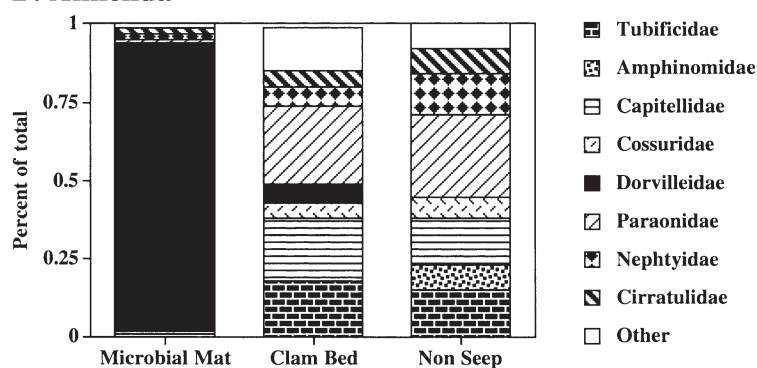


Fig. 5. Macrofaunal (>300 μm) taxonomic composition. (A) Major taxa; (B) annelid family composition

Table 1. Mean (SE) density of macrofauna (>300 µm) per core (54.08 cm² × 10 cm deep) in clam bed (cb) microbial mat (mm), and non-seep (ns) habitats. Differences among microbial mat, clam bed and non-seep habitats were tested with a Kruskal-Wallis test resulting in a chi-square statistic. Probabilities <0.05 are indicated for each taxon. Directions of differences are indicated as (a) ns > cb + mm; (b) mm > cb + ns; (c) cb > ns + mm; (d) ns + mm > cb; (e) cb + ns > mm; Prop.: proportion

Species	Clam bed		Microbial mat		Non-seep		Kruskal-Wallis prob.
	Avg. (SE)	Prop. of total	Avg. (SE)	Prop. of total	Avg. (SE)	Prop. of total	
ANNELIDA							
Oligochaeta							
Tubificidae							
Unid. tubificid	0	0.0	0	0.0	7.00 (5.74)	0.083	0.0525 (a)
<i>Tectidrilus cf. diversus</i>	8.50 (2.65)	0.093	0	0.0	0	0.0	0.0068 (c)
Hirudinea							
Unid. hirudinean	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Polychaeta							
Amphinomidae							
<i>Chloeia pinnata</i>	0.17 (0.17)	0.002	0	0.0	3.75 (1.60)	0.044	0.0034 (a)
Capitellidae							
<i>Mediomastus</i> spp.	9.67 (4.29)	0.106	0.80 (0.49)	0.011	6.75 (2.06)	0.080	
Maldanidae							
Maldanid sp. a	1.33 (0.76)	0.015	0.20 (0.20)	0.003	0.50 (0.29)	0.006	
<i>Nicomache</i> sp. a	0	0.0	0	0.0	0	0.0	
Cossuridae							
<i>Cossura</i> spp.	2.50 (0.56)	0.027	0.60 (0.60)	0.008	3.00 (1.78)	0.035	
Dorvilleidae							
<i>Ophryotrocha platycephale</i>	0	0.0	45.6 (17.82)	0.625	0	0.0	0.0013 (b)
<i>Pseudophryotrocha cf. serrata</i>	0	0.0	5.80 (1.98)	0.079	0	0.0	0.0013 (b)
<i>Ophryotrocha</i> sp. 1	0	0.0	0.60 (0.24)	0.008	0	0.0	0.0302 (b)
<i>Ophryotrocha</i> nr. <i>platycephale</i>	0	0.0	3.40 (1.91)	0.047	0	0.0	0.0318 (b)
<i>Parougia</i> sp. n	1.50 (1.12)	0.016	1.60 (1.03)	0.022	0	0.0	
<i>Exallopus</i> sp. n	1.33 (1.33)	0.015	0	0.0	0	0.0	
Unid. Dorvilleid	0.17 (0.17)	0.002	2.60 (1.63)	0.036	0	0.0	0.0229 (b)
Lumbrineridae							
<i>Lumbrinerid</i> sp. a	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Opheliidae							
<i>Ophelina</i> sp. a	0.17 (0.17)	0.002	0	0.0	0	0.0	
<i>Ophelina acuminata</i>	0	0.0	0	0.0	0	0.0	
Orbiniidae							
<i>Naineris cf. grubei</i>	0.17 (0.17)	0.002	0	0.0	0	0.0	
Paraonidae							
Paraonid sp. a	2.67 (1.85)	0.029	0	0.0	0	0.0	
Paraonid sp. b	2.83 (1.38)	0.031	0	0.0	0.50 (0.50)	0.006	
<i>Levinsenia oculata</i>	5.83 (4.34)	0.064	0	0.0	7.75 (1.80)	0.091	0.022 (a)
<i>Aricidea (Acmira) catherinae</i>	0	0.0	0	0.0	0.50 (0.50)	0.006	
<i>Levinsenia</i> sp. juv.	0.50 (0.34)	0.005	0.60 (0.40)	0.008	3.00 (1.22)	0.035	
Phyllodocidae							
<i>Phyllodoce</i> sp. a	0.33 (0.21)	0.004	0	0.0	0.50 (0.29)	0.006	
Polynoidae							
<i>Harmothoe fragilis</i>	0.50 (0.34)	0.005	0	0.0	0	0.0	
Glyceridae							
Glycerid sp. a	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Goniadidae							
<i>Goniada cf. litorea</i>	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Nephtyidae							
<i>Nephtys cornuta</i>	2.67 (0.88)	0.029	1.60 (0.24)	0.022	5.75 (1.11)	0.068	0.0392 (a)
Nereididae							
<i>Nereis</i> sp. a	0.17 (0.17)	0.002	0	0.0	0	0.0	
Sphaerodoridae							
Sphaerodorid sp. a	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Syllidae							
<i>Syllidae</i> sp. b	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
<i>Sphaerosyllis</i> sp. b	0	0.0	0	0.0	0.25 (0.25)	0.003	
Cirratulidae							
<i>Aphelochaeta</i> sp. h	0.83 (0.83)	0.009	0	0.0	1.75 (1.03)	0.021	
<i>Chaetozone</i> sp. h	0.17 (0.17)	0.002	0	0.0	1.25 (1.25)	0.014	
<i>Aphelochaeta cf. monilaris</i>	0.83 (0.54)	0.009	0	0.0	0.25 (0.25)	0.003	
Unid. cirratulid juv.	0.67 (0.33)	0.007	1.00 (0.45)	0.014	0.50 (0.29)	0.006	
Spionidae							
<i>Prionospio (Minuspio) lighti</i>	0.83 (0.40)	0.009	0	0.0	0	0.0	
Terebellidae							
Terebellid sp. c	0.67 (0.33)	0.007	0	0.0	0	0.0	
<i>Artacama cf. coniferi</i>	0.17 (0.17)	0.002	0	0.0	0	0.0	
Trichobranchidae							
<i>Terebellides</i> sp. a	0.50 (0.22)	0.005	0	0.0	0	0.0	
Ampharetidae							
<i>Ampharete</i> sp. a	0	0.0	0.20 (0.20)	0.003	0	0.0	
Unid. ampharetid (juv)	0	0.0	0.20 (0.20)	0.003	0	0.0	
Unknown terebelliform	0.17 (0.17)	0.002	0	0.0	0.50 (0.50)	0.006	
Unknown spioniform	0	0.0	0	0.0	0.25 (0.25)	0.003	

Table 1 (continued)

Species	Clam bed		Microbial mat		Non-seep		Kruskal-Wallis prob.
	Avg. (SE)	Prop. of total	Avg. (SE)	Prop. of total	Avg. (SE)	Prop. of total	
Nerillidae							
Nerillid sp. a	0.33 (0.21)	0.004	0	0.0	0	0.0	
Flabelligeridae							
<i>Pherusa</i> cf. <i>neopapillata</i>	0.33 (0.21)	0.004	0	0.0	0	0.0	
NEMERTA							
Unid. nemertean	12.17 (3.29)	0.134	1.20 (1.20)	0.016	9.50 (3.23)	0.112	0.0171 (c)
PLATYELMINTHA							
Turbellaria							
Unid. turbellarian	0.17 (0.17)	0.002	1.00 (0.63)	0.014	0	0.0	
ARTHROPODA							
Crustacea							
Amphipoda							
Gammaridea							
<i>Rhachotropis</i> (?)	0	0.0	0.20 (0.20)	0.003	0	0.0	
<i>Bathymedon</i> (?)	0	0.0	0.20 (0.20)	0.003	0	0.0	
<i>Rhachotropis clemens</i>	1.33 (0.98)	0.015	0	0.0	0	0.0	
<i>Harpiniopsis galera</i>	1.50 (0.56)	0.016	0	0.0	0	0.0	
<i>Protomedeia</i> sp. (?)	1.83 (1.27)	0.020	0	0.0	0	0.0	0.0243 (c)
<i>Cephalophoxoides homilis</i>	1.17 (0.65)	0.013	0	0.0	0.25 (0.25)	0.003	
<i>Harpiniopsis</i> spp.	0.83 (0.47)	0.009	0.20 (0.20)	0.003	1.00 (0.71)	0.012	
<i>Harpiniopsis opistomata</i>	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Corophioidea spp.	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
<i>Photis</i> spp.	0.83 (0.54)	0.009	0	0.0	0.50 (0.50)	0.006	
Gammaridea juv.	0.33 (0.21)	0.004	0	0.0	0	0.0	
Unid. gammarideans	0.50 (0.34)	0.005	0	0.0	0	0.0	
<i>Ampelisca usocalae</i>	0.67 (0.49)	0.007	0	0.0	1.25 (0.63)	0.015	
<i>Byblis</i> spp.	0.17 (0.17)	0.002	0	0.0	0.25 (0.25)	0.003	
Caprellidea							
<i>Tritella</i> sp.	0	0.0	0.60 (0.40)	0.008	0	0.0	
Unid. caprellid	0.50 (0.50)	0.005	0	0.0	0	0.0	
Isopoda							
<i>Munnogonium</i> sp. (?)	0.17 (0.17)	0.002	0	0.0	0.50 (0.50)	0.006	
<i>Pleurogonium</i> sp. (?)	0.17 (0.17)	0.002	0.20 (0.20)	0.003	0	0.0	
Cumacea							
<i>Campylaspis</i> sp.	0	0.0	0.40 (0.4)	0.005	0	0.0	
<i>Eudorella pacifica</i>	0.67 (0.33)	0.007	0	0.0	0	0.0	
? <i>Hemilamprops</i> sp. b	0.17 (0.17)	0.002	0	0.0	0	0.0	
Unid. cumacean	0	0.0	0	0.0	1.00 (0.58)	0.012	
Tanaidacea							
<i>Tanaella</i> sp.	1.67 (0.56)	0.018	1.00 (0.63)	0.014	0.25 (0.25)	0.003	
<i>Araphura</i> spp.	5.00 (2.31)	0.055	0.20 (0.20)	0.003	0.75 (0.75)	0.009	0.0361
Unid. tanaid juv.	1.5 (0.56)	0.016	0.60 (0.40)	0.008	1.50 (0.96)	0.018	
Unid. tanaid	0.67 (0.49)	0.007	0	0.0	15 (8.55)	0.177	0.0048 (a)
Mysidacea							
<i>Pseudomma</i> sp.	0.67 (0.49)	0.007	0	0.0	0	0.0	
MOLLUSCA							
Aplacophora							
Unid. aplacophoran	0	0.0	0	0.0	0.25 (0.25)	0.003	
Bivalvia							
<i>Calyptogena</i> sp.	0.83 (0.48)	0.009	0	0.0	0.5 (0.29)	0.006	
<i>Mysella</i> spp.	0	0.0	0.60 (0.40)	0.008	0	0.0	
<i>Rochefortia</i> sp.	0.67 (0.42)	0.007	0	0.0	0	0.0	
<i>Axinopsida serricata</i>	0.50 (0.34)	0.005	0	0.0	0	0.0	
<i>Adontorhina cyclia</i>	0.33 (0.33)	0.004	0	0.0	0	0.0	
Bivalve sp. g	0.17 (0.17)	0.002	0	0.0	0	0.0	
Bivalve sp. bc	0.33 (0.21)	0.004	0.40 (0.24)	0.005	0	0.0	
Unid. bivalve juv.	0	0.0	0.80 (0.20)	0.011	1 (0.41)	0.012	0.0201 (d)
Gastropoda							
<i>Provanna</i> sp.	1.67 (0.92)	0.018	0	0.0	0.75 (0.48)	0.009	
<i>Odostomia</i> sp.	2.83 (0.94)	0.031	0	0.0	1.00 (0.71)	0.012	0.0302 (c)
<i>Astyris permodesta</i>	0	0.0	0.60 (0.60)	0.008	0	0.0	
Scaphopoda							
<i>Cadulus</i> sp.	1.67 (0.49)	0.018	0	0.0	2.00 (0.41)	0.024	0.0052 (e)
<i>Cadulus tolmiei</i>	0	0.0	0	0.0	0.50 (0.29)	0.006	
PORIFERA							
Unid. Porifera	0.17 (0.17)	0.002	0	0.0	0.75 (0.48)	0.009	
ECHINODERMATA							
Ophiuroidea							
Unid. ophiuroid juv.	0	0.0	0	0.0	0.50 (0.50)	0.006	
<i>Ophiosphalma jolliense</i>	1.67 (1.11)	0.018	0	0.0	0	0.0	
Total number of ind.	91.33 (11.93)		73.00 (24.17)		84.75 (9.59)		
Total number of ind. m ⁻²	16891.68 (2207.33)		13502.01 (4470.17)		15674.12 (1774.54)		
Number of species	27.33 (2.11)		12.40 (2.16)		22.25 (1.65)		0.0061 (e)

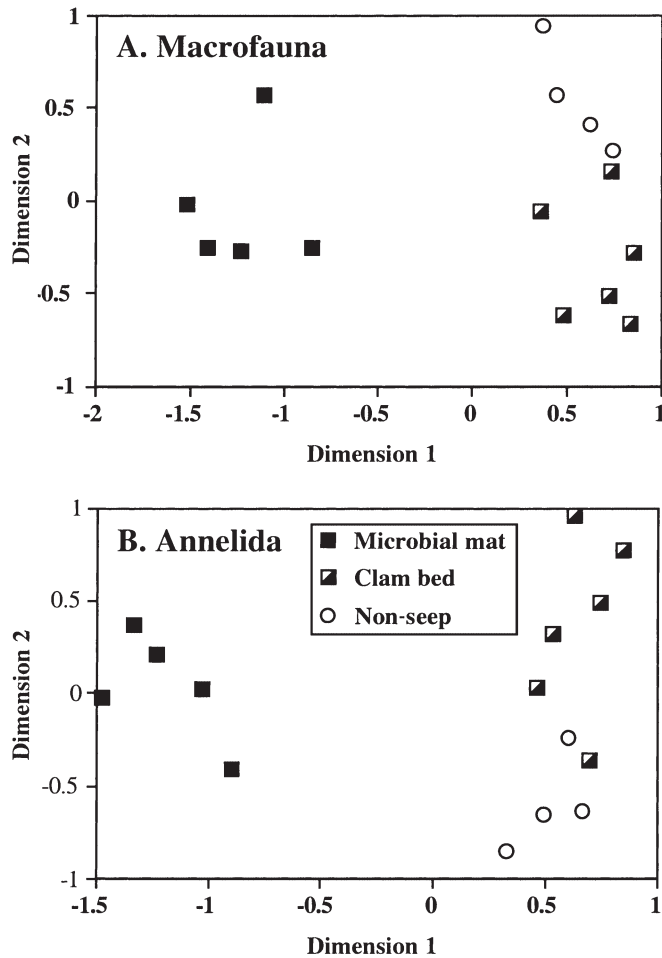


Fig. 6. MDS plot of macrofaunal assemblages in microbial mat, clam-bed and non-seep sediments. (A) Total macrofauna (stress = 0.09); (B) annelids (stress = 0.10)

[$p = 0.0002$] and 10.6% [$p = 0.012$], respectively) than found in microbial mat sediments. Tanaids comprised a greater fraction of the non-seep assemblage than of the clam bed assemblage (19.3 vs 9.6%) ($p = 0.039$).

The microbial mat sediments appear quite distinct from the clam-bed and non-seep habitats when annelids are considered separately at the family level (Fig. 5B). Most annelids in the mat sediments were dorvilleid polychaetes (92%); 87% of the identified dorvilleids belonged to 3 species in the genus *Ophryotrocha*. The only other species to comprise up to 2% of the annelids in the microbial mat habitat was *Nephtys cornuta*. Tubificid oligochaetes, capitellid, and paraonid polychaetes were the most common taxa in the clam bed and non-seep sediments (Fig. 5B; Table 1). No gutless oligochaetes were present at the Eel River site. The tubificid *Tectidrilus cf. diversus* comprised 9.3% of the clam bed macrofauna, but was absent from microbial mat and non-seep sediments. Other tubificid

oligochaetes formed 7.8% of the non-seep macrofauna.

The microbial mat macrofaunal community was highly distinct from those of the clam-bed and non-seep habitats (Fig. 6A; ANOSIM: mat vs clam bed $p = 0.002$; mat vs non-seep $p = 0.008$). The clam bed macrofaunal assemblage also differed from the non-seep community (ANOSIM, $p = 0.010$) (Fig. 6A). However, the microbial mat, clam-bed and non-seep assemblages exhibited similar within-habitat heterogeneity (SIMPER: 41, 35 and 41% similarity, respectively). The annelid assemblage (Fig. 6B), exhibited habitat relationships nearly identical to those seen for total macrofauna (Fig. 6A). However, the clam-bed annelid assemblage exhibited slightly greater heterogeneity among samples (32% within-group similarity) than the mat and non-seep annelid assemblages (both 45% within-group similarity) (SIMPER).

Diversity patterns

Macrofaunal species diversity exhibited habitat-related heterogeneity, consistent with sulfide inhibition of species richness and evenness. The number of macrofaunal species per core was similar in the clam bed (27.3 ± 2.1) and non-seep sediments (22.3 ± 1.7) (Wilcoxon test: $p = 0.195$), but significantly higher than in the microbial mat sediments (12.4 ± 2.2) (Wilcoxon test: $p = 0.007$). For core data pooled by habitat, information ($H'_{\log \text{ base } 2}$) and rarefaction indices of diversity were highest in clam-bed sediments [$H' = 4.99$; $E(S_{100}) = 36.0$], lowest in microbial mat sediments [$H' = 2.50$; $E(S_{100}) = 18.1$], and intermediate within the non-seep sediments [$H' = 4.53$; $E(S_{100}) = 30.0$] (Fig. 7). H' calculated for each core was also significantly lower in microbial mat cores than in clam-bed or non-seep cores (Kruskal–Wallis: $p = 0.004$). Rank 1 dominance (R1D, top-ranked species) was highest for pooled samples in the microbial mat sediments (*Ophryotrocha platycephale* 62%), and lower in the clam-bed (unidentified nemertean 13.4%) and in non-seep sediments (unidentified tanaid 17.7%) (Kruskal–Wallis: $p = 0.023$). However, macrofaunal evenness (J') exhibited no significant differences among habitats (Kruskal–Wallis: $p = 0.156$).

Annelid diversity exhibited patterns similar to those of the total macrofauna. The number of species per core (species richness, S) (Kruskal–Wallis: $p = 0.024$) and H' (Kruskal–Wallis: $p = 0.011$) differed among habitats, with microbial mat annelids exhibiting lowest diversity. Species richness and H' for annelids in clam-bed and non-seep microhabitats did not differ. Annelid evenness (J') (Kruskal–Wallis: $p = 0.422$) and R1D (Kruskal–Wallis: $p = 0.256$) did not differ among habitats.

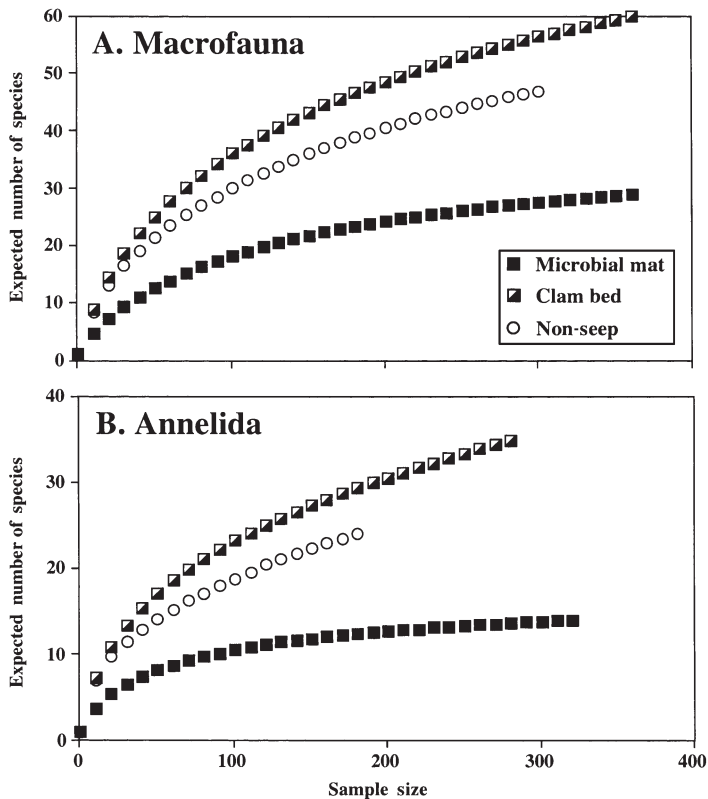


Fig. 7. Rarefaction curves illustrating species richness for pooled samples from microbial mat, clam-bed and non-seep sediments. (A) Total macrofauna; (B) annelids. Species richness values are slightly underestimated because Turbellaria were not identified to species level

Vertical distribution of macrofauna and bacteria

Habitat variation in vertical patterns

Vertical distributions of macrofauna varied among habitats. Macrofauna (mainly dorvilleids) were concentrated in the uppermost sediments in microbial mat cores, with 64% of macrofauna in the 0 to 1 cm layer and 91% in the top 2 cm (Figs. 8A & 9). Only 48 and 40% of macrofauna were present in the top 1 cm of clam bed and non-seep sediments, respectively. A significantly greater fraction of the macrofaunal individuals was found below 2 cm in the non-seep sediments (38% below 2 cm) than in microbial mat sediments (9% below 2 cm) (arcsine percentage test, $p < 0.0001$), but clam-bed patterns (29% below 2 cm) did not differ from those in either of the other microhabitats ($p = 0.162$) (Fig. 8A). Macrofaunal biomass was concentrated in the uppermost 2 cm in all microhabitats (Fig. 8B). Among dorvilleid polychaetes, 46% of individuals were found from 2 to 10 cm in clam-bed sediments; less than 10% occurred below 2 cm in microbial mat sediments (Fig. 9).

Most annelid taxa that tend to dwell deeper in the non-seep sediment column (Paraonidae, Cossuridae, Cirratulidae, Tubificidae) exhibited a slight shallowing of their distribution in clam bed sediments and were restricted to the uppermost 1 cm (or were absent) in microbial mat sediments (Fig. 9). These patterns would be expected if sulfide avoidance controlled vertical distributions. Exceptions were found among capitellid, nephtyid and dorvilleid polychaetes; individuals of these taxa were found down to 5 cm in microbial mat cores (Fig. 9).

Filamentous sulfur bacteria were concentrated in the upper 1 cm in the mat sediments, but were present throughout the entire 10 cm sediment column (Fig. 10). The microbial mat biomass-distribution (Fig. 10B) was shifted upward relative to the filament counts (Fig. 10A), indicating that filaments were larger near the sediment surface. Clam-bed and non-seep sediments also contained living sulfur bacterial filaments concentrated between 1 and 5 cm (Fig. 10).

Sulfide and faunal vertical distributions

The typical relationship between sulfide and animal abundance in this study was L-shaped, with a range of

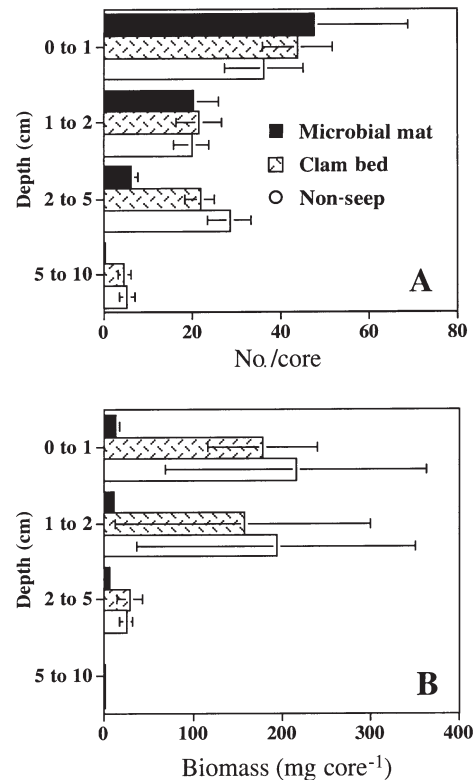


Fig. 8. Vertical distribution of (A) macrofaunal density and (B) macrofaunal biomass. All data are for fauna retained on 300 μ m mesh. Core surface area = 54.1 cm²; depth = 10 cm

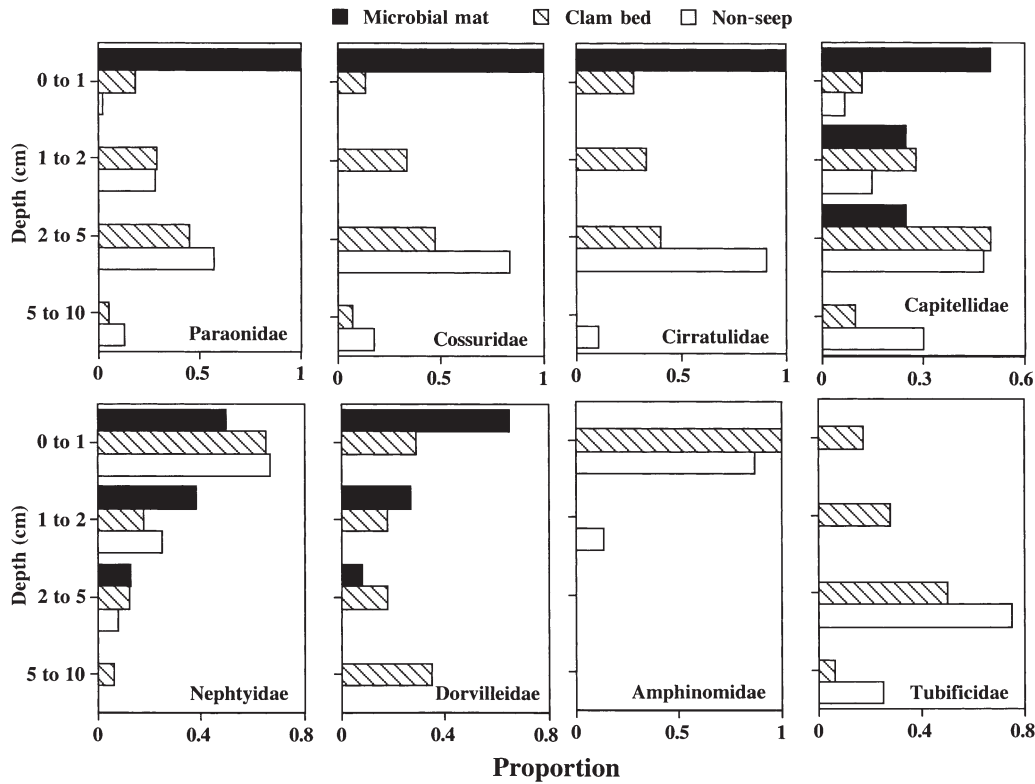


Fig. 9. Vertical distribution of common annelids in microbial mat, clam-bed and non-seep habitats. Data normalized such that values in each vertical fraction represent the proportion of total individuals in each family collected for a specific microhabitat

densities at zero sulfide levels and zero or near-zero densities at higher sulfide levels. This 'L shaped' pattern was observed for cirratulid and paranoid polychaetes, nemertean, amphipods, tanais and gastropods across all settings (Fig. 11). Slight sulfide tolerance was evident among the polychaetes *Nephtys cornuta* and *Mediomastus* spp. (Fig. 11); low densities were observed at 1 to 10 mM sulfide. Counts of total macrofauna, sulfide-oxidizing filamentous bacteria, dorvilleid polychaetes and bivalves (mainly *Vesicomya pacifica*) exhibited highest densities at moderate sulfide levels (1 to 5 mM) and lower densities at 5 to 14 mM sulfide (Fig. 11). Numerous taxa in clam bed cores appeared to reside in the upper 0 to 5 cm of the sediment column, where sulfide concentrations were near zero.

The distribution of individual dorvilleid species in relation to sulfide concentration indicates that different dorvilleid species prefer mat and clam-bed sediments and that, within a microhabitat, some species partition the sediments vertically (Fig. 12). In 1 microbial mat core, *Ophryotrocha platykephale* appeared to prefer sulfide concentrations <4 mM, while 2 other species (*Ophryotrocha* sp. and *Pseudophryotrocha* cf. *serrata*) were most abundant at sulfide concentrations of 4 to 13 mM. In clam-bed sediments, *Parougia* sp. n was present mainly in the upper 5 cm, where sulfide concentrations were low or zero, and *Exallopus* sp. a was found below 5 cm where sulfide concentration was

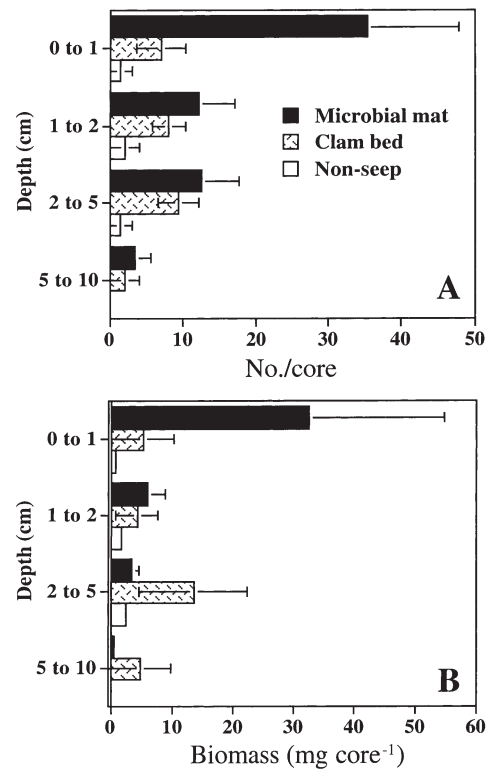


Fig. 10. Filamentous sulfur bacteria. (A) Counts; (B) biomass. Data are for filaments retained on 300 μm mesh. Core surface area = 54.1 cm²; depth = 10 cm

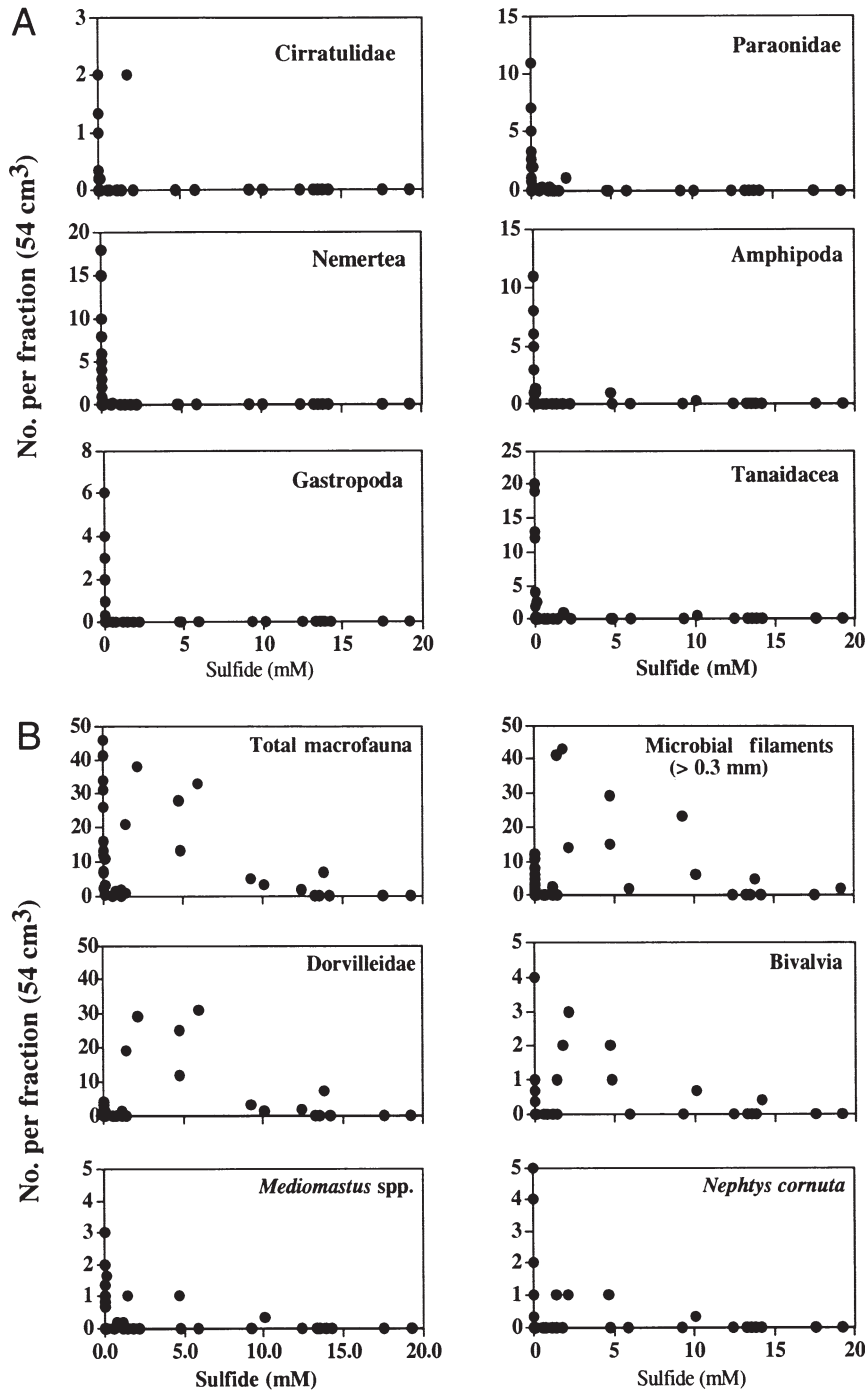


Fig. 11. Scatter-plots of macrofaunal density as a function of sulfide concentration for data from 1 cm thick core sections, all microhabitats combined. (A) Taxa exhibiting sulfide avoidance; (B) taxa exhibiting sulfide tolerance

higher (Fig. 12). Additional fine-scale (<1 cm) measurements of dorvilleid abundance and sulfide concentrations in the same sediments are needed to determine whether these patterns are representative and repeatable at different times and places.

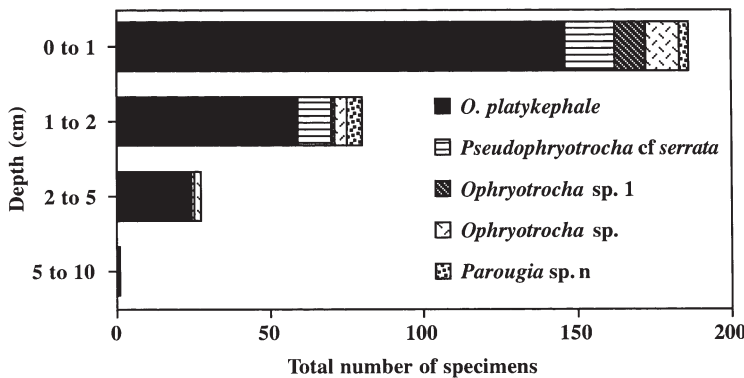
DISCUSSION

Seep habitats

The habitat-scale features (1 to 100 m) of the Eel River upper-slope seeps differ from those of other seep sites in the northeast Pacific. On the Cascadia Margin and in Monterey Bay, methane seep communities display a concentric zonation, with bacterial mats in the center surrounded by different vesicomid clam or solemyid bivalve communities exhibiting different tolerances to sulfide (Sahling et al. 2002, W. Ziebis unpubl. data). On Hydrate Ridge, fluid flow appears to be mainly confined to fewer but more mature and vigorous sites (Tryon et al. 2002), on which large carbonate blocks (chemoherms) have grown over time (Bohrmann et al. 1998). In contrast, the methane seep environments on the Eel River slope form small, visually distinctive patches of *Vesicomya pacifica* communities and mats of filamentous sulfur bacteria, but they are not typically continuous (Figs. 1 & 2). In Monterey Bay, the species *Calyptogena (Vesicomya) pacifica* is associated with lower sulfide concentrations than *C. kilmeri* (Barry et al. 1997). On the Cascadia Margin, *C. pacifica* and *C. kilmeri* communities were encircled by *Acharax* sp. communities along horizontal sulfide gradients (Sahling et al. 2002). *C. kilmeri* and *Acharax* sp. were not observed at the Eel River seeps.

It is possible that highly specific sulfide concentrations, vertical sulfide gradients and fluid expulsion environments are responsible for the formation of the different *Calyptogena kilmeri* and *Acharax* sp. assemblages. The Eel River sulfide concentrations may have been too low in *Vesicomya pacifica* beds and too high in microbial mats for *C. kilmeri* to thrive. It is interesting that the maximum sulfide concentrations measured in Oregon margin clam-bed sediments were similar to those of the Oregon *Beggiatoa* spp. community (Sahling et al. 2002), whereas at the Eel River site, the sulfide concentration measured in intact cores retrieved from the microbial mat and clam-bed habitats

A. Microbial mat



B. Clam bed

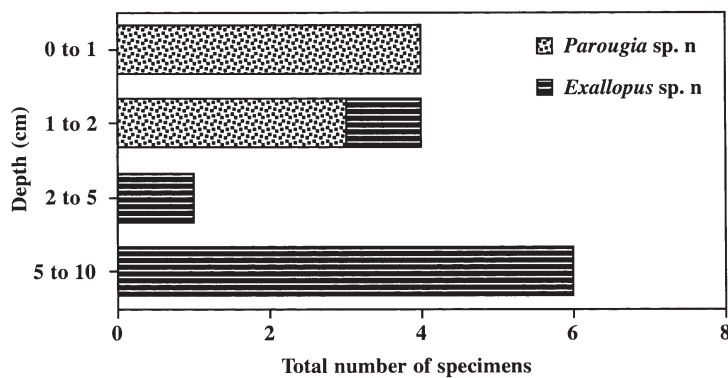


Fig. 12. Vertically resolved plots of dorvilleid species distributions summed across all cores sampled within (A) microbial mat and (B) clam-bed sediments

exhibited order-of-magnitude concentration differences and different vertical distributions (Fig. 4). The sulfide profiles observed by Sahling et al. (2002) in sediments of the Oregon *Calyptogena* (*V. pacifica*) community resemble those observed in *V. pacifica* beds at Eel River in having low sulfide concentrations in the upper 5 cm (Fig. 4), but increased to maximum concentrations of 15 to 25 mM. These values are more characteristic of the Eel River microbial mats, where upward transport of pore fluids occurs (Fig. 3), than the *C. pacifica* beds (maxima of 1 to 2 mM sulfide), where sulfide production occurs locally by sulfate reduction in a defined sediment horizon (~6 to 9 cm) below the zone inhabited by the clams (Ziebis et al. 2002). The 0.1 to 0.2 mM sulfide concentrations present in subsurface sediments of the Oregon *Acharax* sp. assemblage (Sahling et al. 2002) were only slightly higher than we observed in non-seep sediments on the Eel River margin.

Habitat variability

Our sampling revealed a highly patchy system, in which sulfide concentration is correlated with signifi-

cant changes in community structure that occur on small scales both horizontally and vertically. The distinctive nature of the microbial mat assemblage observed in this study is almost certainly due to the attendant high sulfide concentrations (Fig. 4) that result from a high magnitude of vertical upward transport and flux into the overlying water (Fig. 3). However, the limited spatial coverage of our microbial mat measurements prevents generalization to all mat settings in the area. The sulfide-laden fluids that support the high abundances of filamentous sulfur bacteria present at the sediment–water interface are generated by high rates of sulfate reduction supposedly coupled to anaerobic methane oxidation (Boetius et al. 2000, Hinrichs et al. 2000, Orphan et al. 2001). Rates of sulfate reduction and methane oxidation in these Eel River microbial mat sediments were significantly higher than in clam-bed or non-seep sediments (Ziebis et al. 2002).

The dominance of dorvilleid polychaetes in highly sulfidic sediments is not altogether unexpected. Dorvilleids have been observed in sulfidic shallow-water settings including pulp mill and sewage outfalls (Paavo et al. 2000). They are also dominant in hydrothermal mounds in Guaymas Basin (Grassle et al. 1985, Petrecca & Grassle 1987), in sediments surrounding whale falls (Smith et al. 1998, A. Bacco & C. Smith unpubl. data), at seeps on the Oregon margin (Sahling et al. 2002, L. Levin et al. unpubl. data) and in the Gulf of Mexico (C. Robinson et al. unpubl. data), and in oxygen minimum-zone sediments off Mexico (Levin et al. 1991) and northern Chile (L. Levin & V. Gallardo unpubl. data). The dominant species, *Ophryotrocha platykephale*, was first reported from hydrothermal vents (Blake & Hilbig 1990). The co-occurrence of 6 dorvilleid species, 3 of them congeners within the genus *Ophryotrocha*, is highly unusual however, and raises questions about habitat preferences and niche partitioning.

In shallow water, the genus *Ophryotrocha* contains a large number of morphologically similar species that exhibit a broad range of reproductive strategies and are distinguishable with molecular tools (Dahlgren et al. 2001). It is possible that the sulfidic environment found in seep sediments eliminates competitors and predators, providing evolutionary release for dorvilleids. We hypothesize that strong geochemical gradients and unusual microbial food sources associated with methane seeps promote evolutionary differentiation in this genus, and provide the habitat heterogeneity required to support closely related species. Direct gut observations and stable isotopic data suggest that the different dorvilleid species at the Eel River seeps

have widely varying diets, ranging from heterotrophic consumption of phytoplankton-derived organic matter or filamentous sulfur bacteria, to possible possession of symbiotic, sulfide-oxidizing bacteria (L. Levin unpubl. data). Dorvilleid polychaetes at seeps in the Gulf of Alaska and Oregon exhibit exceptionally light $\delta^{13}\text{C}$ signatures (-90‰ , -73‰), suggesting selective consumption of carbon derived from anaerobic methane oxidation (Levin & Michener 2002).

Dorvilleid polychaetes are clearly the most sulfide-tolerant of the taxa present on the Eel River slope (Fig. 11). Only *Vesicomya* sp. and sulfur bacterial filaments exhibited similar tolerance to sulfide concentrations of 5 to 10 mM (Fig. 11). It is not known whether the sulfide tolerance of dorvilleids results from sulfide insensitivity or from well-developed detoxification mechanisms (or both) (Grieshaber & Volkel 1998). The distinct horizontal (habitat) and vertical (microhabitat) distributions of individual dorvilleid species (Fig. 12), suggest that they may be capable of fine-scale selection for specific sulfide conditions.

The general similarity of clam-bed and non-seep macrofauna assemblages observed at the Eel River seeps (Figs. 6 & 7) may reflect their similar hydrologic and porewater geochemical environments down to 4 to 5 cm (Figs. 3 & 4). This is the zone where most of the macrofauna were found and where sulfide concentration was lowest.

A notable difference between the clam-bed and non-seep assemblages was higher overall species richness and greater relative abundance of selected macrofaunal species. We propose that the higher macrofaunal diversity in *Vesicomya pacifica* beds occurs because the clam bed environment represents an ecotone. Clam pumping activity brings oxygen and seawater sulfate down into the sediment, creating an oxygenated habitat in the upper sediments (Fig. 4), and stimulating sulfate reduction and productive sulfide–oxygen interface zones. *V. pacifica* bed sediments support both non-seep taxa, experiencing relatively sulfide-free porewaters in the upper sediment column, and deeper-dwelling species that benefit from sulfide–oxygen interfaces. The sulfide–oxygen interface group includes the oligochaete *Tectidrilus diversus*, as well capitellid, cossurid and paranoid polychaetes, taxa for which $\geq 50\%$ of individuals are found in the 2 to 5 cm fraction (Fig. 9). These groups typically exhibit subsurface density maxima (Blake 1994), but the reasons are not known. Selected species of Capitellidae prefer sulfidic habitats (Gamenick et al. 1998a,b) and grow better in the presence of sulfide (Clough & Lopez 1993, Tsutsumi et al. 2001). In the Eel River clam beds, the interface taxa may be taking advantage of higher microbial biomass in this zone due to increased sulfide oxidation and sulfate

reduction activity (Ziebis et al. 2002), or may merely be more tolerant of sulfides and reside deep in the sediment column to escape predation and competition with other species. Unusually light $\delta^{13}\text{C}$ values have been measured at the Eel River seeps for some of these subsurface, interface taxa (e.g., tubificid oligochaetes ($\delta^{13}\text{C} = -39.7$ to -34.8‰), *V. pacifica* (-36.4 to -36.7‰), the dorvilleid *Parougia* sp. (-35.9‰), a maldanid polychaete (-31.5‰), and the capitellid polychaete *Mediomastus californiensis* (-30.5‰), indicating that they derive nutrition from chemosynthetically fixed carbon sources (Levin & Michener 2002). Benthic foraminifera collected previously from Eel River *Vesicomya pacifica* beds also exhibited high infaunal densities in the upper 5 cm and distinct distributions compared to conspecifics found in adjacent sediments. Differences in carbonate test $\delta^{13}\text{C}$ signatures of these foraminifera also indicated probable ecological and biological differences among co-occurring species (Rathburn et al. 2000).

Vertical patchiness and role of sulfide

Results from this study and previous work (e.g. Torres et al. 2002) suggest that the vertical distribution of sulfide is largely controlled by fluid flow and, where clams are present, by their active pumping. Average flow rates of 10 cm yr^{-1} (Fig. 3) were sufficient to inhibit inflow and extend the sulfide boundary to the sediment surface in the microbial mat habitat (Fig. 4). It is likely that the animal communities we have described reflect geochemical conditions integrated over time. The biological significance of temporal flow variability, particularly the absence of flow for extended periods of time as recorded here at the microbial mat sites, merits further attention.

The distribution of macrofaunal individuals relative to local sulfide concentrations reveals that most taxa avoid sulfide concentrations $>1\text{ mM}$ (Fig. 11). Even in habitats known to contain significant concentrations of sulfide (e.g. *Vesicomya pacifica* beds), most infauna occupy that portion of the sediment column that is relatively sulfide-free. This emphasizes the importance of resolving both animals and porewater geochemistry within the sediment column on fine (1 cm or less) vertical scales when trying to understand the relationships between the two. In previous studies, cores have been taken either for sulfide analyses or for analysis of macrofauna composition, with little attention paid to the vertical distribution of macrofauna. A more complete understanding of how community structure relates to sulfide gradients emerges when fine-scale vertical analyses of sulfide and animal distributions are performed on identical cores.

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