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Journal of Experimental Marine Biology and Ecology 330 (2006) 132 – 150

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Recruitment response of methane-seep macrofauna to sulfide-rich sediments: An in situ experiment

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Received 1 June 2005; received in revised form 12 October 2005; accepted 15 December 2005

Abstract

Hydrodynamically unbiased colonization trays were deployed for 6 months (Oct. 2000 to April 2001) on the northern California margin (Eel R. region; 525 m) to examine macrofaunal colonization rates at methane seeps. The influence of sulfide on recruitment and survival was examined by deploying sediments with and without sulfide added; effect of seep proximity was evaluated by placing trays inside and outside seeps. The trays contained a two-layer system mimicking vesicomyid clam bed habitat geochemistry, with 8–9 mM sulfide in a lower agar layer at the start of the experiment. After 6 month on the seabed, the lower agar layer contained 2–4 mM H_2S. We observed rapid macrofaunal colonization equivalent to 50% of initial non-seep ambient densities. There was no difference in total colonizer densities, number of species, or rarefaction diversity among 3 treatments: (1) controls (no sulfide added) placed outside seeps, (2) trays with sulfide added placed outside seeps and (3) trays with sulfide added placed inside seep patches. Colonization trays with sulfide placed at seeps had different species composition from trays without sulfide place outside seeps; there were more amphipods (non-ampeliscid) and cumaceans in the seep/sulfide treatment and more nemerteans, *Nephtys cornuta* and tanaids in the non-seep/no-sulfide treatment. Outside seeps, annelids comprised < 15% of tray colonists; within seep patches, annelids comprised 5 of the top 10 dominant colonizing taxa (24% of the total). The polychaetes *Mediomastus* sp., *Aphelochaeta* sp., Paraonidae sp., and Nerillidae sp. exhibited significantly higher densities in sulfide additions. Tanaids, echinoderms, and *N. cornuta* exhibited sulfide avoidance. At least 6 dorvilleid polychaete species colonized the experiments. Of these, 4 species occurred exclusively in trays with sulfide added and 80% of all dorvilleid individuals were found in trays with sulfide placed inside seep sediments. Counts of large sulfur bacterial filaments were positively correlated with maximum sulfide concentration in each tray, and with proximity of sulfide to the sediment surface. However, total macrofaunal densities were not correlated with tray sulfide concentrations. As a group, tray assemblages achieved some but not all characteristics of ambient seep assemblages after 6-month exposure on the sea floor. Distinctive colonization patterns at methane seeps contribute to the dynamic mosaic of habitat patches that characterize the eastern Pacific continental margin.

Overall, proximity of seep habitats had at least as great an influence on macrofaunal colonization as tray sulfide concentrations. Taxa characteristic of seep sediments were more likely to settle into trays placed inside rather than outside seep patches. Whether this is due to limited dispersal ability or local geochemical cues remains to be determined.
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Keywords: Colonization; Continental slope; Dorvilleidae; Hydrogen sulfide; Infauna; In situ experiments; Settlement

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doi:10.1016/j.jembe.2005.12.022

1. Introduction

Methane seep sediments on continental margins are highly sulfidic environments. Anaerobic methane oxidation coupled with sulfate reduction yields porewater hydrogen sulfide at mM concentrations, several orders of magnitude higher than typically occurs in non-seep sediments (Boetius et al., 2000; Orphan et al., 2002; Treude et al., 2003). On the northern California margin, porewater sulfide concentrations range from a maximum of around 2 mM in clam bed patches up to 20 mM in bacterial mats (Levin et al., 2003; Orphan et al., 2004; Gieskes et al., 2005). Similar concentrations occur at seeps on the Oregon margin (Sahling et al., 2002). Sulfide is highly toxic to most metazoans (Bagarinao, 1992), but clearly can be tolerated by some organisms (Scott and Fisher, 1995; Fisher, 1998).

Seep sediments often support a mix of seep-specialist and non-seep (ambient) slope infauna (Sibuet and Olu, 1998; Levin, 2005). A high-resolution study of macrofaunal distributions in relation to local (1-cm fraction) sulfide concentration revealed that even in very sulfidic sediments, most seep macrofauna on the Eel R. margin live in the uppermost parts of the sediment column with little or no sulfide. Only a few taxa, such as dorvilleid polychaetes and vesicomyid bivalves, tolerate sulfide concentrations above 1 mM (Levin et al., 2003).

Colonization rates in dynamic deep-sea settings such as seamounts (Levin and DiBacco, 1995), hydrothermal vents (Tunnicliffe et al., 1997), and on continental margins (Snelgrove et al., 1992, 1994, 1996) can be rapid, with 50 to >100% of ambient densities returning to defaunated sediments after 6 months to 2 years. No comparable colonization data have been published for seep sediments. Because geochemistry of seep sediments, and hydrogen sulfide concentrations in particular, are thought to govern the distribution of seep infauna (Sibuet and Olu, 2002; Sahling et al., 2002, 2003; Levin et al., 2003), it was deemed likely that geochemical cues may influence settlement or survival of colonizing seep taxa. Among non-symbiont bearing macrofauna, the genera *Capitella* and *Ophryotrocha* have been shown to survive or grow well in the presence sulfide (Tsutsumi et al., 2001; Levin et al., 2003). The genus *Capitella* can be abundant at sulfidic shallow-water vents (Gamenick et al., 1998a,b) and methane seeps (Levin et al., 2000), but there is debate about whether hydrogen sulfide is a settlement cue (Cuomo, 1985) or is sublethally toxic (Dubilier, 1988) for this genus. Other porewater constituents such as ammonium or oxygen can induce avoidance behavior in settling invertebrate larvae (Marinelli and Woodin, 2002).

The goal of this study was to examine factors influencing colonization rates of macrofauna at N. California methane seeps (525 m water depth). In particular we examined (1) the influence of porewater sulfide, and (2) the effect of seep proximity, on the composition and abundance of colonizers. We investigated the extent to which sulfide provides a settlement attractant or avoidance cue for seep macrofauna by deploying colonization trays (consisting of a sediment-filled central cup with surrounding broad collar) with and without sulfide added. Trays with sulfide were placed inside and outside seep patches to examine how proximity to seep sediments influences colonization in the presence of sulfides.

We tested the null hypotheses that colonization of trays should be independent of (a) sulfide additions and (b) placement inside vs outside seep sediment patches. Our alternative hypotheses were that seep specialists should settle in greater numbers in sediments (a) with sulfide added and (b) situated within seep patches. Based on observations of animal distributions at the Eel River methane seeps (Levin et al., 2003), we expected dorvilleid polychaetes and vesicomyid bivalves to show the greatest positive responses to sulfide presence. Based on observations of higher diversity in clam bed than ambient sediments at Eel R. (Levin et al., 2003), we hypothesized that trays with sulfides added should exhibit higher species richness than those without. The study also examined which taxa present in background sediments were rapid colonizers on the continental margin.

2. Methods

2.1. Colonization trays

Research was carried out on the northern California margin (USA) at 525 m off shore of the mouth of the Eel River (47.1°N 135.7°W). The site, its geochemical features, biological habitats, and infaunal communities of seep (vesicomyid clam bed, bacterial mat) and non-seep sediments are described in Levin et al. (2000, 2003) and Gieskes et al. (2005). Colonization trays were deployed from the R/V Thompson using ROVs. Trays were placed on the sea floor in October 2000 by the ROV Jason I, and recovered in April 2001 by the ROV Oceanic Explorer. We used long-baseline, transponder-guided navigation with Jason I on the deployment cruise (accurate to within 1–10 m) and short-baseline navigation (accurate to within 50 m) on the recovery cruise with the Oceanic Explorer. The colonization tray design, hydrodynamic properties, and deployment and

recovery mechanisms are described in Snelgrove et al. (1992) and Levin and DiBacco (1995). The colonization tray apparatus consisted of an 11.4-cm diameter cup (9 cm deep) lined with nitex mesh (20 μm), surrounded by a flat Delrin nylon collar that was 40 cm in diameter. The collar was designed to prevent water flow from scouring the sediments in the central cup (Snelgrove et al., 1992). A hole for the sediment cup was created in the seabed by taking a tube core (8.3 cm diameter) or box core (15 × 15 cm). When the cores were successful (about 50% of the time) they provided a time 0 record of ambient infaunal densities and composition (to 10 cm depth in the sediment column). The colonization trays were nestled into the sediment such that the cup sediment surface and collar lay flush with surrounding sediments. Water-tight deployment and recovery lids were used to prevent loss of tray sediments during transport to and from the ship. Trays were deployed and recovered 4 at a time by the ROV; we used a gear elevator (similar to the one depicted in Levin and DiBacco, 1995) to transport trays to and from the ship.

2.2. Sediment/sulfide treatments

For the colonization experiments, the contents of the sediment cups of the colonization trays were prepared so as to simulate sulfide gradients similar to those previously measured at seep habitats in Monterey Bay during June 2000 (Rathburn et al., 2003). Tray sediment cups contained a two-layer sediment system, in which the lower 4-cm fraction consisted of agar. The upper 5 cm consisted of defaunated slope sediments (0–10 cm fraction) collected from non-seep sediments in the Eel R. region by box core from the RV Sproul in June 1998 and frozen at −20 °C until use in 2000.

To create the sulfide treatments, a 4-cm thick layer of agar (1%) was prepared with the addition of sodium sulfide crystals (936 mg/L agar solution) to yield a concentration of ~12 mM (78 mg/L=>1 mM). Single sodium sulfide crystals were rinsed with water, dried carefully with Kimwipes, and if necessary crushed into smaller pieces. 936 mg were weighed immediately and transferred into 500 mL of filtered sea water that was purged with nitrogen gas for 30 min to remove oxygen. The solution was kept anoxic while being stirred. A second volume of 500 mL seawater was also purged with nitrogen gas, 10 g of Agar (Bacto) were added and the solution was heated until boiling. The sulfide solution was also slowly heated then added to the agar solution under constant stirring and under a stream of nitrogen gas at the point when the agar solution started to turn clear. The jar containing the sulfide agar solution was placed in a glove box under inert gas (nitrogen). The sulfide–agar solution was then poured into 2 cm-high petri dishes (150 × 25 mm), and allowed to cool. The petri dishes were purged additionally with nitrogen gas before they were closed, sealed with electric tape and double bagged in nitrogen-purged Ziploc bags for storage in the cold (4 °C) until use at sea. The control treatments were prepared in the same way without the addition of sodium sulfide to the agar.

At sea the agar was cut in a glove box under nitrogen atmosphere with a specially prepared 'cookie cutter' (11.4 cm diameter), and two 2-cm layers were combined in the bottom of each colonization tray cup. After addition of defaunated sediment over the agar, colonization trays were stored for 10–30 min in the cold room (4 °C) in small buckets filled with oxygen-free seawater (purged with nitrogen) until they were attached to the tray rims for deployment. Sulfide and oxygen microprofiles were measured in the ship's cold room (4 °C) in selected cups before deployment. Six control (non-sulfide) and 12 sulfide treatments were prepared.

Sediment cups were lowered into small plastic buckets filled with deoxygenated seawater on the gear elevator, just prior to deployment. Trays were deployed in groups of three. One sulfide tray was placed inside a seep patch, a second sulfide tray and a control (no sulfide) tray were placed in the surrounding non-seep sediment area, 5 m apart from each other and within 10 m of the seep patch. This was repeated in 6 different areas (blocks) over a region about 400 × 250 m; each block was marked with a syntactic foam marker and *x*–*y* coordinates were noted. Five of the seep patches receiving trays were vesicomyid clam beds (hereafter referred to as clam beds). A sixth was a bacterial mat.

2.3. Ambient sediment data

Tube cores (8.3 cm diameter × 10 cm deep) of ambient (background) sediment were collected initially in the vicinity of sediment trays during deployment (Oct. 2000). We ultimately obtained 15 background cores (6 in clam beds, 5 in microbial mats and 4 in non-seep sediments). We obtained 10 ambient sediment cores in April 2001. Five were taken in vesicomyid clam beds and 1 in a microbial mat, adjacent to trays. Six non-seep cores were taken, one in each block, in sediments located between paired non-seep colonization trays. However, 2 failed.

2.4. Tray processing

All 18 colonization trays were recovered 6 months after deployment. Immediately after retrieval, the central sediment cups (still attached to the collars) were lowered into beakers containing low-oxygen seawater in the cold room (4 °C). Prior to further processing, microprofiles of oxygen and hydrogen sulfide were measured immediately in all trays in the cold room (see methods below). After microprofiling, the bottom panel was removed from the sediment cups and sediments were extruded (from below) and sectioned at 1-cm intervals to 5 cm then at 5–7 and 7–9 cm intervals. Sediments were preserved in 10% buffered formalin and seawater until they could be sorted for macrofauna. In the laboratory, sediments were sieved on a 300-μm mesh. Retained animals and microbial filaments were sorted from sediments at 12× magnification under a dissecting microscope. The sulfur bacteria present at this site are relatively robust and large (mm's in length). While some may have fragmented in handling, filament counts can indicate relative abundance of sulfide oxidizing bacteria among treatments. Animals were identified to the lowest possible taxonomic classification, usually the species level. Even when precise names could not be assigned, an effort was made to distinguish among species to analyze diversity. The tray cup surface area was 102 cm^2. Tube cores of ambient sediments collected in October 2000 and April 2001 were extruded, sectioned and processed as described above. Microprofiles of oxygen and sulfide were made for ambient cores collected in October 2000. In April 2001, microprofiles were generated only for the recovered trays; time limitations prevented profiling of ambient sediments.

2.5. Microprofiling

Oxygen and H_2S microgradients were measured in ambient cores and H_2S was measured in intact colonization trays immediately after retrieval by using amperometric microelectrodes. Oxygen was measured by Clark-type microelectrodes with a built-in reference and a guard cathode (Jørgensen and Revsbech, 1985, 1989). The electrodes had a sensing tip of 20 to 40 μm, a stirring sensitivity of <2% and a 90% response time of ≤1 s. H_2S 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 by preparing a stock solution of S^{-2} (100 mM). The H_2S microsensors respond linearly over a certain range (i.e. 0–2 and 2–20 mM). A stock solution of hydrogen sulfide (i. e. 100 mM) was prepared from dissolving Na_2S in N_2-flushed 0.1 M NaOH in a closed container. A subsample of the stock solution was fixed with Zn–acetate and subsequently the exact concentration of the 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 7.0). Oxygen was removed from this buffer by bubbling with an oxygen-free inert gas (e. g. N_2) before aliquots were transferred to gas-proof containers with rubber stoppers. A suitable reductant (e.g. Ti(III) Cl; in a 10% HCl solution) was added to a final concentration of 1 mM. The signal zero is obtained by immersing the sensor tip into the calibration buffer. Further calibration points were prepared by injecting suitable amounts of the sulfide stock solution into the calibration vials with a micro-syringe. Two calibration curves were prepared, one for a concentration range between 0 and 2 mM and a second for higher concentrations from 2 to 20 mM. Typically 5 standards were prepared for each calibration curve. The principle of the amperometric H_2S sensors is that H_2S from the environment will penetrate through the sensor tip membrane into the alkaline electrolyte, where the HS ions formed are oxidized immediately by ferricyanide, producing sulfur and ferrocyanide. The sensor signal is generated by re-oxidation of ferrocyanide at the anode in the tip of the sensor (Jeroschewsky et al., 1996). The sensor detects the partial pressure of H_2S gas, which is only one component of the total sulfide equilibrium system. For the calculation of total sulfide concentrations $[S_{tot}^{-2}]$ (Jeroschewsky et al., 1996), it is necessary to know the pH. Thus, when H_2S gradients were measured, pH was determined along the same profile using long pH combination needle electrodes (Diamond General), which were connected to a high impedence mV-meter. Profiles presented in this paper are shown as total sulfide concentrations and our use of the term 'sulfide' refers to total sulfide. As H_2S sensors are sensitive to temperature, it is necessary to perform calibrations and measurements at the same temperature. Profiles made at sea were measured in the cold room at 3 °C, the in situ temperature. The oxygen and sulfide gas sensors were purchased from UNISENSE, Denmark.

For profiling, the sensors were attached to a micromanipulator mounted on a heavy stand. Signals were amplified by a picoammeter (Unisense PA 2000) and data were recorded directly on a computer. Measurements were performed in vertical increments of 250 μm for oxygen and 1 mm for H_2S.

2.6. Statistical analyses

Sediment trays and ambient tube cores were analyzed for macrofaunal abundance (density), species number, composition, vertical distribution and rarefaction diversity ($E(s_{100})$). Data were tested for normality (rarely found) and log transformed prior to parametric analyses. We used 1-way ANOVA to test for tray treatment effects on bacteria filament and macrofaunal total abundance and on each species. We applied paired *t* tests (pairing within blocks) to make two balanced comparisons where $n=6$: (1) trays with sulfide placed inside vs outside seep patches and (2) trays with vs without sulfide placed outside seep patches. Due to the low number of replicates and potential spatial variability among blocks, we felt a paired approach would better resolve our questions. Multidimensional scaling, ANOSIM and SIMPER (Primer Software V.5) were used to examine similarity of assemblages colonizing the different treatments, and the similarity of colonizer assemblages to ambient sediment seep and non-seep assemblages. Error terms presented with the mean are standard error unless indicated otherwise. We set $\alpha=0.10$ for this study, due to the low number of replicate samples [a persistent problem in deep-sea research] and high spatial variability inherent to the system. However, all *P* values are presented so the readers may draw their own conclusions about significance.

3. Results

3.1. Geochemical microprofiles

3.1.1. Unmanipulated sediments

Repeated measurements made in cores from non-seep, clam bed and microbial mat sediments showed consistent profiles of oxygen and hydrogen sulfide (Levin et al., 2003). Oxygen penetrated 3 to 4 mm deep into non-seep and clam-bed sediments whereas in the microbial mats oxygen was already consumed at the sediment–water interface (Fig. 1A). The sulfide profiles of the microbial mat core suggested a strong hydrogen sulfide flux from below; hydrogen sulfide reached the sediment/water interface and was present at very high concentrations (14–19 mM) throughout the upper 10 cm of the cores. In the clam beds, no sulfide was detected in the upper 4 cm. Below this zone, hydrogen sulfide increased with depth to a maximum concentration of up to 2 mM at ~9 cm depth, indicating a well-defined zone of local sulfide production by sulfate reduction. In non-seep sediments, hydrogen sulfide was absent or occurred at a low concentration (<0.2 mM) (Fig. 1B).

3.1.2. Colonization trays

Profiling of four colonization trays ~1 h prior to deployment revealed that the sulfide concentration in the agar layer was about 8–9 mM at deployment, with pH between 7 and 8.

Sulfide profiles generated for each tray after 6 months on the seafloor reflected loss of sulfide in the 'with sulfide' treatments and generation of small amounts of sulfide by organic matter degradation in the non-seep, defaunated sediments. However, sulfide concentrations remained significantly higher in trays with sulfide added ($t_{14}=3.63$; $P=0.003$; Figs. 2A, 3). A maximum concentration of 3–4 mM was observed in most of the sulfide addition trays at collection time, whereas maximum concentrations were $\ll 1$ mM sulfide in the control (non-addition) trays (Fig. 3). Sulfide diffused upward, but more slowly than expected, so that much of the upper 3–4 cm remained relatively free of sulfide (Fig. 3). Vertical profiling revealed that sulfide concentrations of 50, 100, and 200 μM were present closer to the sediment surface in sulfide-addition vs non-addition trays (Fig. 2B).

3.2. Sulfur bacteria

Counts of bacterial filaments after ~6-month exposure were not significantly different in colonization tray sediments with sulfide added (9.8±2.9 filaments 102 cm^{-2} inside seeps and 11.8±4.7 102 cm^{-2} outside seeps) to those without sulfide (5.8±2.9 filaments per tray) ($F_{2,13}=1.256$; $P=0.323$). Average filament counts in sulfide trays were higher than those in ambient non-seep sediments in Oct. 2000 (2.7 102 cm^{-2}), but were not different than those in natural clam beds or bacterial mat habitats in April or October ($F_{8,42}=4.478$; $P=0.0009$). While we did not observe differences among sulfide treatments, the likely influence of H_2S on the abundance of bacterial filaments is indicated by a positive correlation of filament counts with the maximum sulfide concentration in each tray ($r^2=0.50$, $P=0.051$), and a negative correlation of counts with the depth in the sediment at which H_2S concentrations of 50 μM were observed ($r^2=0.53$; $P=0.005$) (Fig. 2C). Counts of bacteria in trays were not correlated with macrofaunal colonizer abundance ($r^2=0.004$, $P=0.80$).

3.3. Macrofauna

3.3.1. Background seep and non-seep macrofauna

Total macrofaunal densities were similar in non-seep (15,678±1775 ind. m^{-2}), seep-clam bed (16,897

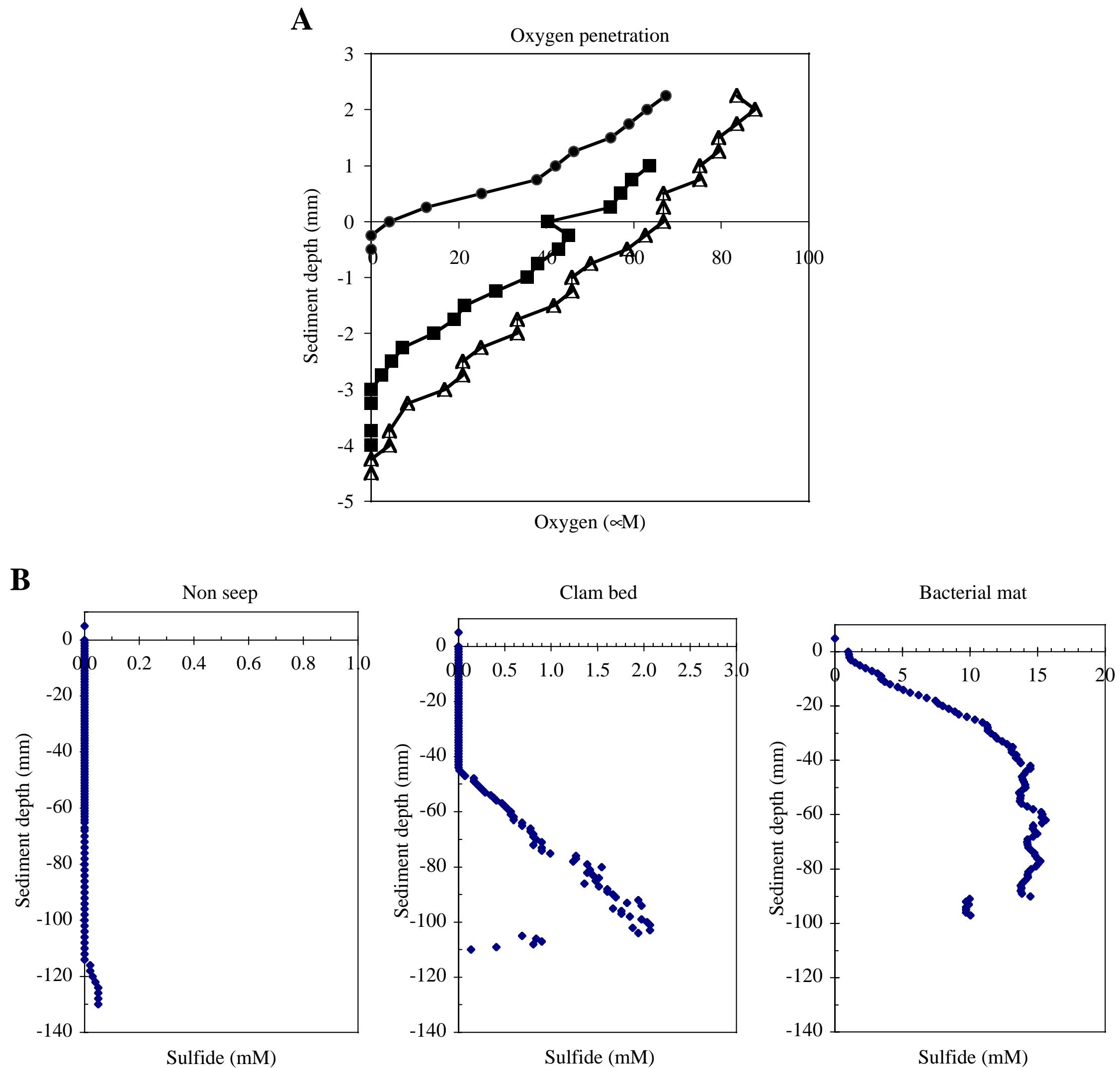

Fig. 1. A. Typical oxygen profiles measured in sediment cores retrieved from non-seep (triangles), clam-bed (squares), and microbial-mat (circles) habitats at methane seeps on the Eel River Margin. B. Typical hydrogen sulfide profiles for the same habitats.

±2208 ind. m^{-2}) and seep-microbial mat sediments (13,505±4470 ind. m^{-2}) at the start of the experiment (Oct. 2000) ($F_{2,14}$=0.966; P=0.408) (Fig. 4). Six months later, when colonization trays were collected, non-seep densities (12,071±1083 ind. m^{-2}) exhibited no significant change. However, April 2001 clam bed and microbial mat densities were significantly higher than April non-seep densities or any October densities ($F_{5,26}$=7.043, P=0.0005, Fig. 4). Clam bed densities in April increased by more than 3 times October values to 45,621±7853 ind. m^{-2}. Only one bacterial mat sample was collected in April 2001, and that also had very high densities (62,160 ind. m^{-2}).

3.3.2. Colonization tray macrofauna

3.3.2.1. Abundances. Total densities of macrofaunal colonizers attained about 50% of October background seep and non-seep values during the 6-month exposure period. However, average macrofaunal densities pooled across all trays (6827±753 ind. m^{-2}) did not differ significantly from those in background seep or non-seep sediments at the start of the experiment or from those in non-seep sediments at the end of the experiment. Colonization trays exhibited significantly lower macrofaunal densities than the ambient seep sediments in April 2001 ($F_{8,42}$=8.318; P=<0.0001), achieving only 15% of clam bed macrofaunal densities (Fig. 4).

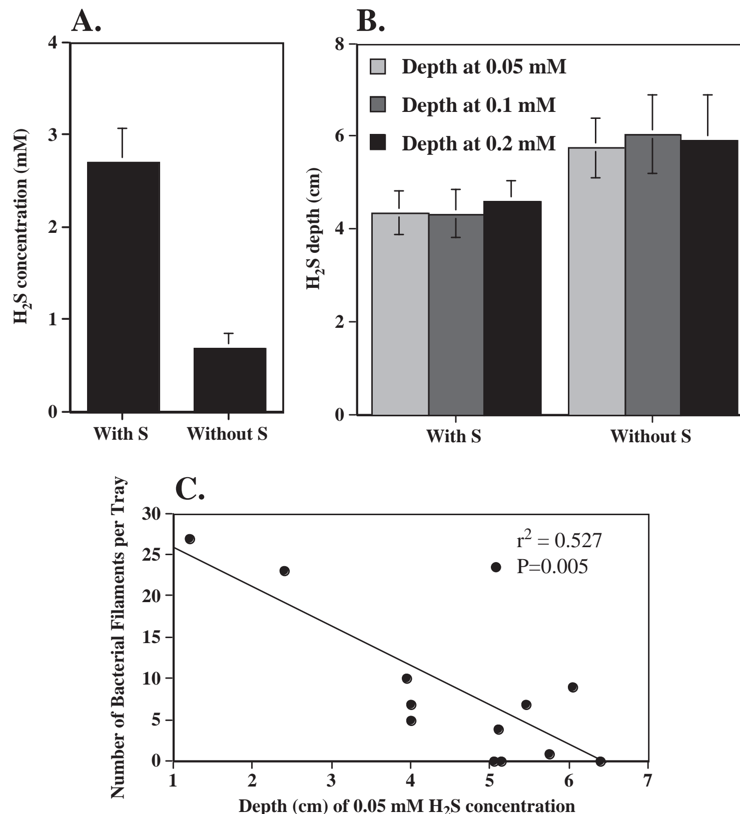

Fig. 2. Sulfide data for 3 colonization tray treatments after 6-month deployment at 525 m on the Eel R. margin. $n = 6$ trays per treatment. A. Mean (±1 SE) maximum sulfide concentration (mM) upon recovery. Initial concentrations were 8–9 mM in trays with sulfide added. B. Mean depth within the sediment column at which sulfide concentration reached 50 μM. C. Counts of filamentous sulfur bacteria (>300 μm) regressed against depth of the 50-μM sulfide horizon. Sulfur bacteria are more abundant when sulfide occurs shallower in the tray.

3.3.2.2. Composition. There was much consistency among tray treatments in the top colonizing taxa (Table 1). The most abundant taxa colonizing the trays were nemerteans (27.6%), peracarid crustaceans including the amphipods *Rhachotropis clemens* (7.2%) and *Ampelisca unsocalae* (5.4%), cumaceans (6.6%), tanaids (6.5%), and the polychaetes *Nephtys cornuta* and *Chloeia pinnata* (each 5.7%). Together these six taxa comprised 64% of the tray colonists. Other frequently encountered colonists included turbellarians (3.5%), scaphopods (2.2%), *Calyptogena* sp. (2.0%), *Ophryotrocha platykephale* (1.8%), and *Mediomastus* sp. (1.6%). Nearly all of the most abundant colonizers were common in natural sediments at the start or end of the experiment (Table 1).

Overall, polychaetes were less abundant and formed a smaller proportion of the total in the tray assemblages (27%) than in ambient assemblages in October 2000 (57%) and April 2001 (46%). Outside of seep patches, annelids typically comprised <15% of the total macrofaunal tray colonists; the nephtyid *N. cornuta* and the amphinomid *C. pinnata* were the primary annelid representatives in these sediments. Among trays placed inside seeps, annelids comprised 5 of the top 10 taxa and represented 24% of the total colonists (Table 1). Peracarid crustaceans had lower densities (1965 ind m^{-2}) but represented a larger fraction of the total macrofauna (28.8%) in colonization trays than in October (2874 ind m^{-2}; 18.6%) and April (3533 ind m^{-2}; 10.4%) ambient assemblages. Molluscs were equally well represented in trays and October 2000 samples (7.1% and 7.6%, respectively), but were a much larger fraction of the April 2001 fauna (34.1%) (Table 2).

3.3.2.3. Vertical distribution. Most of the colonizing individuals were present in the uppermost 3 cm of the trays. Only 5.8% of the colonists (33 taxa) were found below 3 cm and only 1.8% below 5 cm (17 taxa). The fraction of macrofaunal taxa residing at sediments depths >3 cm in trays was 8.0%, 6.5% and 3.5% in seep/sulfide, non-seep/sulfide and non-seep/no sulfide

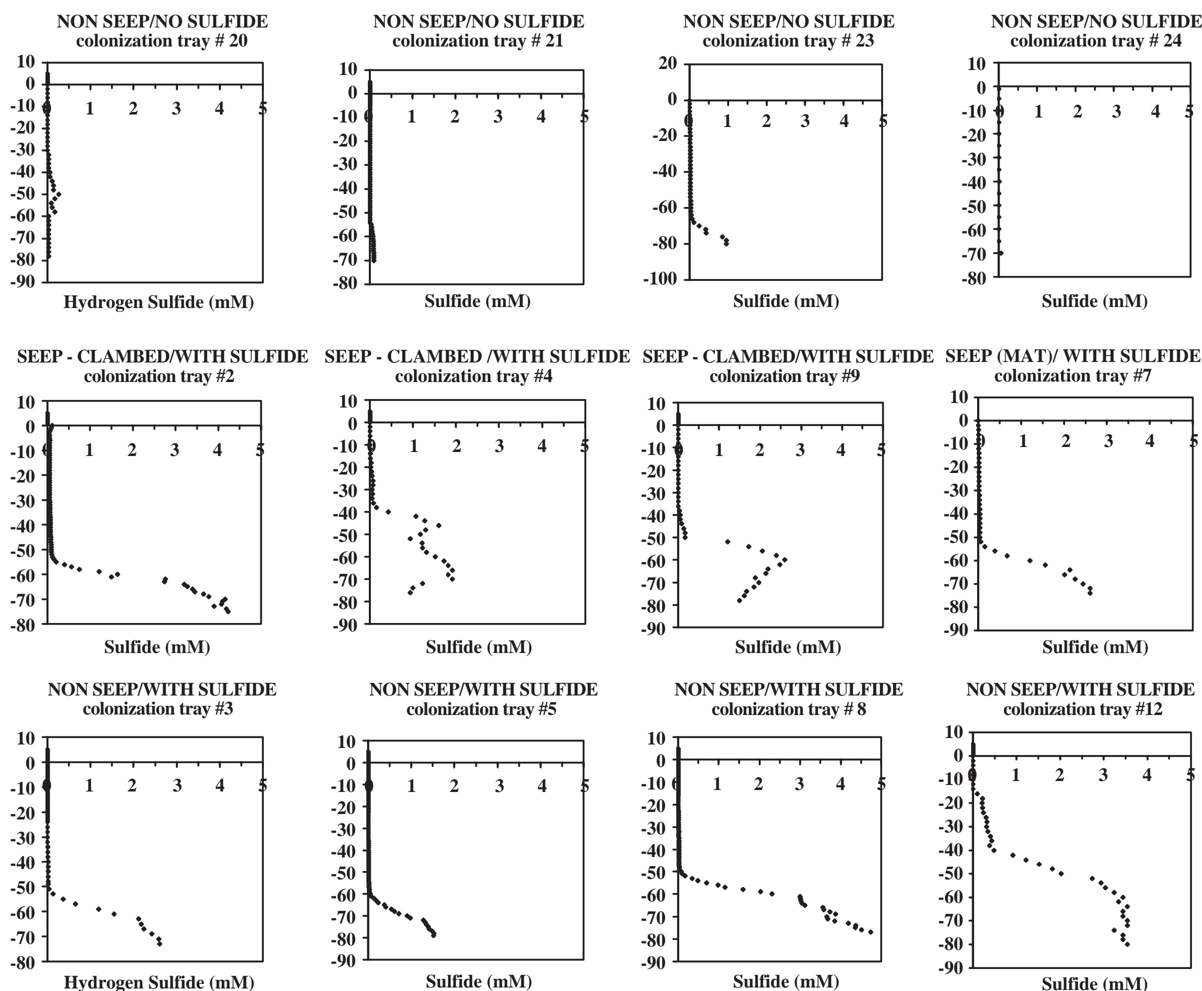

Fig. 3. Representative sediment sulfide profiles for 3 colonization tray treatments after 6-month deployment at 525 m on the Eel R. margin. Top row: Control sediments without sulfide, placed outside seeps. Middle row: Sediments with sulfide, placed inside seeps (clam bed or microbial mat) and Bottom row: Sediments with sulfide, placed outside seeps. Twelve of 18 total trays are shown.

treatments, respectively. The twenty-three individuals residing at depths of 5–9 cm in agar laden with sulfides included dorvilleid polychaetes (9 indiv. belonging to 3 species), tanaids (4 individuals), gammarid amphipods (3 individuals), capitellid (2 individuals) and phyllodocid polychaetes (2 individuals), and a single isopod, cumacean, cossurid polychaete and nemertean.

3.3.3. Sulfide influence: sulfide vs no sulfide addition

Total macrofaunal densities in colonization trays did not differ among the two sulfide treatments (sulfide vs no sulfide) for trays placed outside seeps (Paired t test, $t_5=0.787$, $P=0.467$; two tailed). Paired t tests for 19 taxonomic groups revealed few taxa to be significantly more abundant in one sulfide treatment or the other (outside seeps only). Higher densities in sulfide treatments were observed for the annelids *Mediomastus* sp. ($t_5=-1.58$; $P=0.087$, one tailed), *Aphelochaeta* sp. H ($t_5=-1.58$, $P=0.087$), Paraonidae ($t_5=-2.36$, $P=0.038$; one tailed), and Nerillidae ($t_5=-1.58$, $P=0.087$. *N. cornuta* exhibited the reverse trend (sulfide avoidance; $t_5=-1.87$, $P=0.061$). In evaluating all trays, we noted that the cirratulid *Aphelochaeta* sp. H; the spionid *Prionospio* (*Minuspio*) *lighti* and nerillid polychaetes colonized only the trays with sulfide added (Table 2). When all 18 trays were considered in an unpaired t test (those inside and outside seep patches irrespective of block), we found sulfide preference by the Nerillidae ($t_{16}=1.95$, $P=0.069$), and sulfide avoidance among echinoderms ($t_{16}=-2.31$, $P=0.036$) and tanaids ($t_{16}=-2.27$, $P=0.038$) (Fig. 5). Densities of

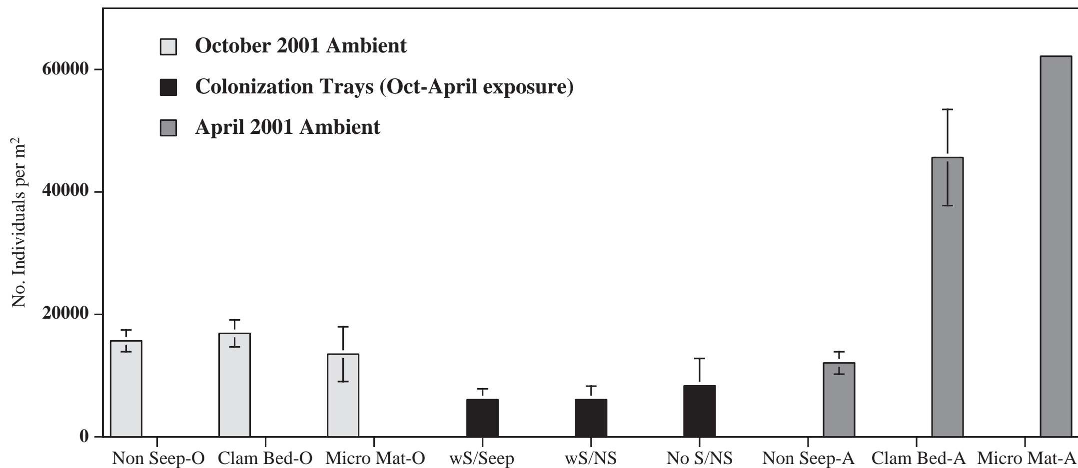

Fig. 4. Mean (±1 SE) macrofaunal density (>300 μm) in natural sediments sampled in October 2000 and April 2001 (non-seep, clam bed and microbial mat) and in colonization tray sediments. Trays contained agar and defaunated sediments as one of 3 treatments: no sulfide added—placed outside seeps; sulfide added—placed outside seeps; sulfide added—placed inside seeps (clam bed or microbial mat).

colonizing amphipods, cumaceans, gastropods and scaphopods were unaffected by sulfide additions (paired tests, $P>0.10$).

3.3.4. Habitat influence: seep vs non-seep patches

For colonization trays containing sulfide, total macrofaunal colonizer densities did not differ among the two placement locations (inside vs outside seeps). This is consistent with the absence of seep vs non-seep density effects in natural macrobenthos in October 2000 (Levin et al., 2003). However, higher densities were observed among sulfide addition trays placed at seeps than in non-seep sediments for the oligochaete *Tectidrilus diversus* (paired *t* test, $t_5=-1.72$, $P=0.073$) and the spionid polychaete *Prionospio* (*Minuspio*) *lighti* ($t_5=-2.24$; $P=0.038$). Seep avoidance was observed among Paraonidae ($t_5=2.74$, $P=0.020$, paired *t* test one tailed). When all 18 trays were considered in an unpaired design (6 in seep sediments, 12 outside seep patches), three taxa were found to exhibit greater abundance within seep patches: Dorvilleidae ($t_{16}=1.76$, $P=0.098$), *Mediomastus* sp. ($t_{16}=1.98$, $P=0.066$) and Oligochaeta ($t_{16}=2.51$, $P=0.023$) (Fig. 5). Two taxa, *Ophryotrocha platykephale* and *Prionospio* (*Minuspio*) *lighti* colonized only trays in seep patches and none of the 12 trays in non-seep patches.

At least 6 dorvilleid polychaete species colonized the trays. Of these, 4 species occurred exclusively in trays with sulfide added: *O. platykephale*, *Ophryotrocha* sp. 1, *Pseudophryotrocha* cf. *serrata*, and *Parougia* sp. nov. (Fig. 6). Eighty percent of all dorvilleid individuals were found in trays with sulfide placed inside seep sediments.

3.3.5. Multivariate analyses: treatment comparisons, similarity to ambient sediments

Taken together, macrofaunal assemblages present in colonization trays with sulfide added were similar whether placed in seep vs non-seep habitats (MDS: $R=0.124$, $P=0.115$). Macrofaunal assemblages present in colonization trays placed in non-seep habitats were unaffected by the presence or absence of sulfide ($R=-0.024$, $P=0.578$). However, trays with sulfide placed at seeps had different assemblages from trays without sulfide place outside seeps ($R=0.22$, $P=0.019$) (Fig. 7). There were more amphipods (non-ampeliscid) and cumaceans in the seep/sulfide treatment and more nemerteans, *N. cornuta* and tanaids in the non-seep/no-sulfide treatment (SIMPER). In combination these taxa accounted for 50% of the individuals present in these two treatments.

The assemblages in each of the colonization tray treatments differed from background samples taken in October (ANOSIM: bacterial mat—$P=0.002$; clam bed $P=0.002$; and non-seep—$P=0.005$) and in April (clam bed — $P=0.002$, non-seep—$P=0.005$). In general, colonization trays had more nemerteans, while Oct. background seep samples had more *O. platykephale*, tanaids, and numerous polychaetes. April background seep samples had more *Odostomia* sp., *Provanna* sp., *Exallopus* sp., tanaids, amphipods and *Mediomastus* sp. (SIMPER, Table 1). Tray assemblages were more similar to October than April background samples (Fig. 7, Tables 1, 2). Trays placed outside seeps had assemblages most similar to October non-seep samples. Trays placed inside seeps (with sulfide) had assemblages most similar to the Oct. clam bed samples (Tables 1, 2).

Table 1
Ten top-ranked taxa in non-seep, seep sediments and colonization tray treatments, and their percent representation ( )

Rank	Background — non-seep		Background — clam bed	
	Oct-00	Apr-01	Oct-00	Apr-01
1	Tanaidacea (17.7)	Tanaidacea (11)	Nemerteans (13.3)	*Odostomia* sp. (34.3)
2	Nemerteans (11.2)	Nemerteans (10.6)	*Mediomastus* sp. (10.6)	Dorvilleidae (20.4)
3	*Levinsenia oculata* (9.1)	*Mediomastus* sp. (8.1)	*Tectidrilus* cf *diversus* (9.3)	*Provanna* sp. (11.2)
4	Tubificidae (8.3)	Dorvilleidae (6.9)	*Levinsenia oculata* (6.4)	Nemerteans (5.2)
5	*Mediomastus* sp. (8.0)	*Nephtys cornuta* (5.8)	Tanaidacea sp. (6.2)	Tanaidacea (3.6)
6	*Nephtys cornuta* (6.8)	*Levinsenia oculata* (5.2)	*Odostomia* spp. (3.1)	*Protomedeia* sp. (3.2)
7	*Chloeia pinnata* (4.4)	Paraonidae unid (4.2)	Gammarid sp. (3.1)	*Calyptogena* sp. (3.2)
8	*Levinsenia* spp. (3.5)	*Aricidea catherinae* (3.6)	Paraonid sp. b (3.1)	*Tectidrilus diversus* (3.0)
9	*Cossura* spp. (3.5)	*Calyptogena* sp. (3.1)	*Nephtys cornuta* (2.9)	Turbellarian (2.0)
10	*Cadulus* spp. (2.4)	*Harpiniopsis* sp. (2.7)	Paraonid sp. a (2.9)	*Mediomastus* sp. (2.1)
Total percent	74.90%	61.20%	61.10%	89.20%

Rank	Background — microbial mat	
	Oct-00	Apr-01
1	*Ophryotrocha platykephale* (62.5)	*Ophryotrocha platykephale* (25.9)
2	*Pseudophryotrocha* cf *serrata* (8.0)	*Ophryotrocha* sp. 1 (16.1)
3	*Ophryotrocha* nr *platykephale* (4.7)	*Pseudophryotrocha* cf *serrata* (15.2)
4	Unid dorvilleid (3.6)	*Parougia* sp. nov (13.7)
5	*Nephtys cornuta* (2.2)	Gastropod sp. af (6.6)
6	*Parougia* sp. nov. (2.2)	*Calyptogena* sp. (3.7)
7	Unid bivalve (1.6)	Unid. *Ophryotrocha* (3.0)
8	Tanaidacea (1.6)	Unid. Ampharetidae (2.7)
9	Nemertean (1.6)	Nemertean (2.4)
10	Turbellarian (1.4)	*Ampelisca* sp. (2.1)
Total percent	89.30%	89.40%

Rank	Colonization trays — April 2001		
	No S/outside seep	W/ S outside seep	With S/inside seep
1	Nemerteans (46.4)	Nemerteans (21.3)	Nemerteans (15.1)
2	Tanaidacea (8.8)	*Chloeia pinnata* (10.2)	Gammarid amphipods (12.9)
3	*Nephtys cornuta* (7.1)	Cumacean (10.0)	Cumaceans (6.5)
4	*Ampelisca unsocalae* (5.9)	Tanaidacea (5.4)	*Nephtys cornuta* (5.9)
5	Cumacea (3.3)	Ampeliscid amphipod (5.4)	Tanaidacea (5.4)
6	*Rhachotropis clemens* (3.1)	*Rhachotropis clemens* (5.4)	*Ophryotrocha platykephale* (5.4)
7	Turbellarian (2.8)	Turbellarian (4.5)	*Chloeia pinnata* (5.4)
8	Scaphopoda (2.0)	*Nephtys cornuta* (4.0)	Ampeliscid amphipod (4.9)
9	*Chaetozone* sp. (1.6)	*Calyptogena* spp. (3.5)	*Tectidrilus diversus* (4.0)
10	*Chloeia pinnata* (1.6)	Scaphopoda (3.0)	*Mediomastus* sp. (3.8)
Total percent	82.50%	72.80%	69.30%

The percentage of macrofauna accounted for by the top 10 taxa is given below the list.

Because few studies have examined temporal stability of seep infaunal assemblages, it is informative to compare the Oct. 2000 and April 2001 background communities. There was no temporal change in the background non-seep assemblages (ANOSIM, $P=0.314$) or microbial mat assemblages ($P=0.167$, but $n=1$ in 2001), but a significant shift was observed in background clam bed assemblages ($P=0.002$), largely due to 2001 increases in densities of the gastropods *Odostomia* sp. and *Provanna* sp. and of the dorvilleid polychaete *Exallopus* sp. (SIMPER, Tables 1, 2).

3.3.6. Diversity

Species diversity evaluated as the number of species present and with rarefaction analysis (expected species number relative to sample size), was similar in all three colonization tray treatments ($F_{2,17}=1.05$, $P=0.373$; Fig. 8). We found on average 19.2, 17.2 and 16.3 species per tray in seep/sulfide, non-seep/sulfide and non-seep/no sulfide treatments, respectively, suggesting a trend towards diversity enhancement by seep influence. $E(s_{100})$ values ranged from 17.8 to 22.5 in colonization tray treatments. Tray rarefaction diversities were

Table 2
Mean density (1 SE) and proportion of macrofauna in ambient and colonization tray sediments. Data are normalized to number per 54.08 cm^2

Species	October 2000 microbial mat	October 2000 microbial mat	October 2000 clam bed	October 2000 clam bed	Oct. 2000 non-seep	Oct. 2000 non-seep	April 2001 clam bed	April 2001 clam bed	April 2001 bacteria mat	April 2001 bacteria mat	April 2001 non-seep	April 2001 non-seep	CT with sulfide seep	CT with sulfide seep	CT with sulfide non-seep	CT with sulfide non-seep	CT without sulfide non-seep	CT without sulfide non-seep
	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total		Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total
Annelida																		
Oligochaeta																		
Unid tubificid	0.000	0.000	0.000	0.000	7 (5.74)	0.083	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Tectidrilus cf. *diversus*	0.000	0.000	8.50 (2.66)	0.093	0.000	0.000	7.60 (2.98)	0.031	0.000	0.000	0.000	0.000	1.33 (0.71)	0.040	0.09 (0.09)	0.003	0.09 (0.09)	0.002
Hirudinea																		
Unid. Hirudinean	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Polychaeta																		
Chloeia pinnata	0.000	0.000	0.17 (0.17)	0.002	3.75 (1.60)	0.044	0.40 (0.24)	0.002	0.000	0.000	0.75 (0.75)	0.011	1.77 (0.53)	0.054	3.36 (1.93)	0.103	0.71 (0.33)	0.016
Capitella spp.	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mediomastus spp.	0.80 (0.49)	0.011	9.67 (4.29)	0.106	6.75 (2.06)	0.080	3.80 (1.77)	0.015	0.000	0.000	5.25 (0.85)	0.080	1.24 (0.72)	0.038	0.26 (0.18)	0.008	0.09 (0.09)	0.002
Maldanid sp. a	0.20 (0.20)	0.003	1.33 (0.76)	0.015	0.50 (0.29)	0.006	0.000	0.000	0.000	0.000	1.25 (0.63)	0.019	0.09 (0.09)	0.003	0.18 (0.11)	0.005	0.000	0.000
Nicomache sp. a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Unid maldanid (juv)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Cossura spp.	0.60 (0.60)	0.008	2.50 (0.56)	0.027	3 (1.78)	0.035	2 (0.45)	0.008	0.000	0.000	1.00 (0.58)	0.015	0.000	0.000	0.35 (0.26)	0.011	0.000	0.000
Ophryotrocha platykephale	45.60 (17.82)	0.625	0.000	0.000	0.000	0.000	0.000	0.000	87.000	0.259	0.000	0.000	1.77 (1.56)	0.054	0.18 (0.18)	0.005	0.000	0.000
Pseudophryotrocha cf. *serrata*	5.80 (1.98)	0.079	0.000	0.000	0.000	0.000	0.80 (0.49)	0.003	51.000	0.152	2 (2)	0.031	0.18 (0.18)	0.005	0.000	0.000	0.000	0.000
Ophryotrocha sp. 1	0.60 (0.24)	0.008	0.000	0.000	0.000	0.000	4 (1.10)	0.016	54.000	0.161	0.000	0.000	0.97 (0.97)	0.030	0.000	0.000	0.000	0.000
Ophryotrocha nr. *platykephale*	3.40 (1.91)	0.047	0.000	0.000	0.000	0.000	0.60 (0.60)	0.002	3.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Parougia spp.	1.60 (1.03)	0.022	1.50 (1.12)	0.016	0.000	0.000	0.80 (0.58)	0.003	46.000	0.137	1.75 (0.75)	0.027	0.18 (0.11)	0.005	0.09 (0.09)	0.003	0.09 (0.09)	0.002
Exallopus sp. n	0.000	0.000	1.33 (1.33)	0.015	0.000	0.000	45.20 (28.26)	0.183	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ophryotrocha nr. *bifida*	0.000	0.000	0.000	0.000	0.000	0.000	4.20 (1.96)	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pinniphitime sp. n.	0.000	0.000	0.000	0.000	0.000	0.000	3.80 (3.32)	0.015	1.000	0.003	0.50 (0.50)	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Ophryotrocha cf. *puerilis*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Unid *Ophryotrocha*	0.000	0.000	0.000	0.000	0.000	0.000	0.40 (0.40)	0.002	10.000	0.030	0.50 (0.50)	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Parougia oregonensis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dorvillea (*Schistomeringos*) sp. a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.18 (0.18)	0.004

Polychaeta																		
Pettiboneia brevipalpata Bannlon	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Unid. dorvilleid	2.60 (1.63)	0.036	0.17 (0.17)	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.18 (0.11)	0.005	0.09 (0.09)	0.002
Lumbrinerid spp.	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	0.20 (0.20)	0.001	0.000	0.000	0.50 (0.29)	0.008	0.09 (0.09)	0.003	0.09 (0.09)	0.003	0.000	0.000
Fauveliopsid sp. a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.18 (0.18)	0.005	0.000	0.000	0.000	0.000
Ophelina sp. a	0.000	0.000	0.17 (0.17)	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ophelina cf. *farallonensis*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Ophelina acuminata	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.09 (0.09)	0.003	0.000	0.000	0.09 (0.09)	0.002
Naineris cf. *grubei*	0.000	0.000	0.17 (0.17)	0.002	0.000	0.000	0.60 (0.40)	0.002	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Paraonid sp. a	0.000	0.000	2.67 (1.86)	0.029	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.26 (0.12)	0.008	0.09 (0.09)	0.003	0.18 (0.18)	0.004
Paraonid sp. b	0.000	0.000	2.83 (1.38)	0.031	0.50 (0.50)	0.006	0.000	0.000	0.000	0.000	1.75 (1.75)	0.027	0.000	0.000	0.62 (0.29)	0.019	0.000	0.000
Levinsenia oculata	0.000	0.000	5.83 (4.34)	0.064	7.75 (1.80)	0.091	0.000	0.000	0.000	0.000	4.25 (1.55)	0.065	0.000	0.000	0.000	0.000	0.18 (0.18)	0.004
Levinsenia gracilis	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Levinsenia spp.	0.60 (0.40)	0.008	0.50 (0.34)	0.005	3 (1.22)	0.035	1.20 (0.97)	0.005	0.000	0.000	2 (2)	0.031	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.002
Unid. paraonid	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	1.000	0.003	2.75 (1.60)	0.042	0.000	0.000	0.000	0.000	0.000	0.000
Aricidea (*acmia*) *catherinae*	0.000	0.000	0.000	0.000	0.50 (0.50)	0.006	0.000	0.000	0.000	0.000	2 (2)	0.031	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Phyllodoce spp.	0.000	0.000	0.33 (0.21)	0.004	0.50 (0.29)	0.006	0.20 (0.20)	0.001	0.000	0.000	0.25 (0.25)	0.004	0.26 (0.18)	0.008	0.53 (0.27)	0.016	0.53 (0.27)	0.012
Halosydna spp.	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Harmothoe imbricata	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Harmothoe fragilis	0.000	0.000	0.50 (0.34)	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Glycerid sp. a	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Glycera branchiopoda	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Glycinde armigera	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Nephtys cornuta	1.60 (0.24)	0.022	2.67 (0.88)	0.029	5.75 (1.11)	0.068	0.80 (0.37)	0.003	0.000	0.000	3.25 (0.95)	0.050	1.95 (0.47)	0.059	1.33 (0.47)	0.041	3.19 (1.17)	0.071
Nereis spp.	0.000	0.000	0.17 (0.17)	0.002	0.000	0.000	2.20 (1.11)	0.009	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Unid. nereidid (juv)	0.000	0.000	0.000	0.000	0.000	0.000	1.00 (1)	0.004	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.09 (0.09)	0.002
Goniada cf. *litorea*	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sphaerodorid sp. a	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Unid. syllid	0.000	0.000	0.17 (0.17)	0.002	0.25 (0.25)	0.003	1.80 (0.49)	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.18 (0.18)	0.005	0.53 (0.53)	0.012
Sphaerosyllis sp. b	0.000	0.000	0.000	0.000	0.25 (0.25)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.18 (0.11)	0.005	0.000	0.000

(continued on next page)

Table 2 (*continued*)

Species	October 2000 microbial mat	October 2000 microbial mat	October 2000 clam bed	October 2000 clam bed	Oct. 2000 non-seep	Oct. 2000 non-seep	April 2001 clam bed	April 2001 clam bed	April 2001 bacteria mat	April 2001 bacteria mat	April 2001 non-seep	April 2001 non-seep	CT with sulfide seep	CT with sulfide seep	CT with sulfide non-seep	CT with sulfide non-seep	CT without sulfide non-seep	CT without sulfide non-seep
	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total		Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total
Exogone (*Parexogone*) sp. a	0.000	0.000	0.000	0.000	0.000	0.000	2 (2)	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hesionid spp.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.75 (0.48)	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Aphelochaeta sp. h	0.000	0.000	0.83 (0.83)	0.009	1.75 (1.03)	0.021	1.20 (0.58)	0.005	0.000	0.000	0.25 (0.25)	0.004	0.35 (0.18)	0.011	0.18 (0.11)	0.005	0.000	0.000
Chaetozone sp. h	0.000	0.000	0.17 (0.17)	0.002	1.25 (1.25)	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.003	0.000	0.000
Chaetozone gracilis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.75 (0.75)	0.011	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Chaetozone cf. *setosa*	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.26 (0.27)	0.008	0.000	0.000	0.71 (0.38)	0.016
Aphelochaeta cf. *monilaris*	0.000	0.000	0.83 (0.54)	0.009	0.25 (0.25)	0.003	0.20 (0.20)	0.001	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Unid. cirratulid (juv)	1.00 (0.45)	0.014	0.67 (0.33)	0.007	0.50 (0.29)	0.006	0.20 (0.20)	0.001	0.000	0.000	0.50 (0.50)	0.008	0.09 (0.09)	0.003	0.53 (0.27)	0.016	0.000	0.000
Tharyx spp.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.18 (0.11)	0.004
Prionospio (*Minuspio*) *lighti*	0.000	0.000	0.83 (0.40)	0.009	0.000	0.000	2 (1.14)	0.008	0.000	0.000	1.00 (1)	0.015	0.26 (0.12)	0.008	0.000	0.000	0.000	0.000
Laonice nuchala	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.002
Terebellid spp.	0.000	0.000	0.67 (0.33)	0.007	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.75 (0.48)	0.011	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Artacama cf. *coniferi*	0.000	0.000	0.17 (0.17)	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000
Unid terebellid	0.000	0.000	0.000	0.000	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Trichobranchid sp. a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.002
Octobranchus sp. a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Terebellides sp. a	0.000	0.000	0.50 (0.22)	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Terebellides cf. *reishi*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.50 (0.50)	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Ampharete sp. a	0.20 (0.20)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.26 (0.18)	0.008	0.000	0.000	0.000	0.000
Mugga wahebergi	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Amphisamytha cf. *bioculata*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.18 (0.18)	0.005	0.000	0.000	0.18 (0.11)	0.004
Lysippe labiata	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Unid. ampharetid	0.20 (0.20)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	9.000	0.027	0.25 (0.25)	0.004	0.09 (0.09)	0.003	1.33 (0.53)	0.041	0.18 (0.18)	0.004
Pherusa cf. *neopapillata*	0.000	0.000	0.33 (0.21)	0.004	0.000	0.000	1.80 (0.97)	0.007		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Annelida																		
Polychaeta																		
Brada villosa	0.000	0.000	0.000	0.000	0.000	0.000	1.00 (1)	0.004		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Unid. flabelligerid (juv)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Unknown terebelliform polychaete	0.000	0.000	0.17 (0.17)	0.002	0.50 (0.50)	0.006	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Unknown spioniform polychaete	0.000	0.000	0.000	0.000	0.25 (0.25)	0.003	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nerillid sp. a	0.000	0.000	0.33 (0.21)	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.75 (0.75)	0.011	0.26 (0.12)	0.008	0.26 (0.12)	0.008	0.000	0.000
Nemertea																		
Unid. nemertean	1.20 (1.20)	0.016	12.17 (3.29)	0.133	9.50 (3.23)	0.112	10.20 (4.14)	0.041	8.000	0.024	7.25 (3.64)	0.111	4.96 (1.43)	0.151	6.99 (2.47)	0.214	20.89 (11.75)	0.464
Turbellaria																		
Unid. turbellarian	1.00 (0.63)	0.014	0.17 (0.17)	0.002	0.000	0.000	6.60 (1.03)	0.027	4.000	0.012	0.50 (0.50)	0.008	1.06 (0.43)	0.032	1.50 (0.92)	0.046	1.24 (0.94)	0.028
Crustacea																		
Amphipoda																		
Gammaridea			8.67															
Non-ampeliscid gammarids	0.60 (0.40)	0.008	(3.01)	0.095	2.25 (1.11)	0.027	12.40 (4.01)	0.050	4.000	0.006	4 (1.87)	0.061	5.40 (1.58)	0.164	2.30 (0.53)	0.070	2.12 (0.66)	0.047
Ampeliscid gammarids	0.000	0.000	0.83 (0.54)	0.009	1.50 (0.50)	0.018	0.40 (0.40)	0.002	7.000	0.021	1.50 (0.96)	0.023	1.59 (0.39)	0.049	1.86 (0.65)	0.057	2.65 (0.83)	0.059
Caprellidea																		
Unid. caprellid	0.60 (0.40)	0.008	0.50 (0.50)	0.005	0.000	0.000	0.20 (0.20)	0.001	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.18 (0.11)	0.005	0.18 (0.11)	0.004
Isopoda																		
Unid. isopod	0.20 (0.20)	0.003	0.33 (0.33)	0.004	0.50 (0.50)	0.006	0.40 (0.24)	0.002	6.000	0.018	1.00 (0.71)	0.015	0.35 (0.35)	0.011	0.35 (0.11)	0.011	0.44 (0.16)	0.010
Cumacea																		
Unid. cumacean	0.40 (0.40)	0.005	0.83 (0.48)	0.009	1.00 (0.58)	0.012	1.40 (0.93)	0.006	0.000	0.000	0.50 (0.50)	0.008	2.12 (0.80)	0.065	3.27 (1.86)	0.100	1.50 (0.54)	0.033
Tanaidacea																		
Unid. tanaid	1.80 (1.20)	0.025	8.83 (3.81)	0.097	17.50 (10.33)	0.206	8 (4.66)	0.032	2.000	0.006	7.75 (2.95)	0.119	1.77 (0.56)	0.054	1.77 (0.47)	0.054	3.98 (1.22)	0.088
Mysidacea																		
Pseudomma spp.	0.000	0.000	0.67 (0.49)	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mollusca																		
Aplacophora																		
Unid. aplacophoran	0.000	0.000	0.000	0.000	0.25 (0.25)	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.53 (0.36)	0.016	0.26 (0.18)	0.006
Bivalvia																		
Calyptogena spp.	0.000	0.000	0.83 (0.48)	0.009	0.50 (0.29)	0.006	11.20 (6.83)	0.045	12.000	0.036	2 (2)	0.031	0.62 (0.16)	0.019	1.15 (0.76)	0.035	0.62 (0.40)	0.014
Non-Calyptogena bivalves	1.80 (0.37)	0.025	2 (1.03)	0.022	1.00 (0.41)	0.012	0.000	0.000		0.000	0.50 (0.50)	0.008	0.000	0.000	0.26 (0.18)	0.008	0.35 (0.35)	0.008
Gastropoda																		
Provanna spp.	0.000	0.000	1.67 (0.92)	0.018	0.75 (0.48)	0.009	16.60 (16.60)	0.067	0.000	0.000	0.000	0.000	0.35 (0.18)	0.011	0.53 (0.27)	0.016	0.88 (0.49)	0.020
Gastropod sp. ac	0.000	0.000	0.000	0.000	0.000	0.000	0.000 86	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.003	0.35 (0.11)	0.008
Odostomia sp.	0.000	0.000	2.83 (0.95)	0.031	1.00 (0.71)	0.012	(21.68)	0.349	4.000	0.012	1.00 (0.71)	0.015	0.62 (0.29)	0.019	0.000	0.000	0.18 (0.18)	0.004

(continued on next page)

Table 2 (*continued*)

Species	October 2000 microbial mat	October 2000 microbial mat	October 2000 clam bed	October 2000 clam bed	Oct. 2000 non-seep	Oct. 2000 non-seep	April 2001 clam bed	April 2001 clam bed	April 2001 bacteria mat	April 2001 bacteria mat	April 2001 non-seep	April 2001 non-seep	CT with sulfide seep	CT with sulfide seep	CT with sulfide non-seep	CT with sulfide non-seep	CT without sulfide non-seep	CT without sulfide non-seep
	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total		Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total	Mean (SE)	Prop. of total
Astyris permodesta	0.60 (0.60)	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gastropod sp. af	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	22.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Scaphopoda																		
Cadulus spp.	0.000	0.000	1.67 (0.49)	0.018	2.50 (0.29)	0.029	0.000	0.000	0.000	0.000	0.75 (0.48)	0.011	0.53 (0.34)	0.016	0.97 (0.32)	0.030	0.88 (0.35)	0.020
Echinodermata																		
Asteroidea																		
Unid. Asteroidea	0.000	0.000	0.000	0.000	0.000	0.000	0.60 (0.60)	0.002	0.000	0.000	0.25 (0.25)	0.004	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Ophiuroidea																		
Ophiuroid spp.	0.000	0.000	1.67 (1.12)	0.018	0.50 (0.50)	0.006	0.60 (0.24)	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.002
Porifera																		
Unid. porifera	0.000	0.000	0.17 (0.17)	0.002	0.75 (0.48)	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cnidaria																		
Anthozoa																		
Unid. Anthozoa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.09 (0.09)	0.003	0.000	0.000
Hydrozoa																		
Unid. hydrozoa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Unid. cnidarian	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.26 (0.27)	0.008	0.000	0.000
Miscellaneous vermiforms	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Unknown Phylum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Unknown ab	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09 (0.09)	0.003	0.000	0.000	0.000	0.000
Total macrofauna	73 (24.17)		91.33 (11.94)		84.75 (9.59)		246.60 (42.45)		338.000		65.25 (9.89)		32.83 (4.13)		32.74 (6.05)		45.05 (16.12)	

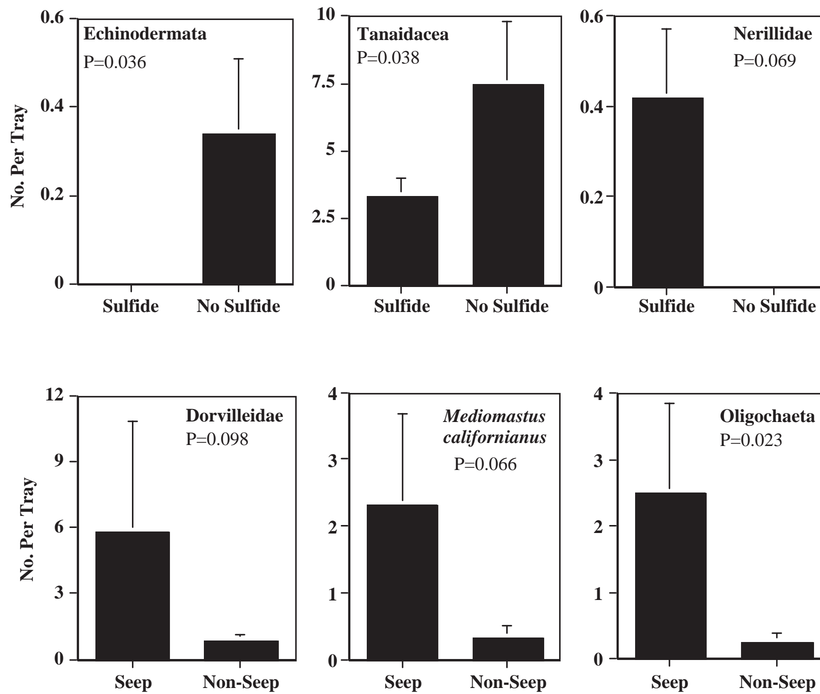

Fig. 5. Mean density (±1 SE) of selected taxa colonizing trays that exhibited sulfide avoidance (echinoderms, tanaids) or attraction (Nerillidae) and seep habitat attraction (Dorvilleidae, *Mediomastis* sp., Oligochaeta). Top row: $n=12$ trays with sulfide, $n=6$ trays without sulfide. Bottom row: $n=6$ trays inside seep patches, $n=12$ trays outside seep patches.

comparable to October background microbial mat ($E(s_{100})=16.1$) and non-seep sediments ($E(s_{100})=24.6$) and April clam bed sediments ($E(s_{100})=21.0$), but lower than in non-seep sediments in April 2001($E(s_{100})=33.8$) and clam bed sediments in October ($E(s_{100})=28.3$) (Fig. 8).

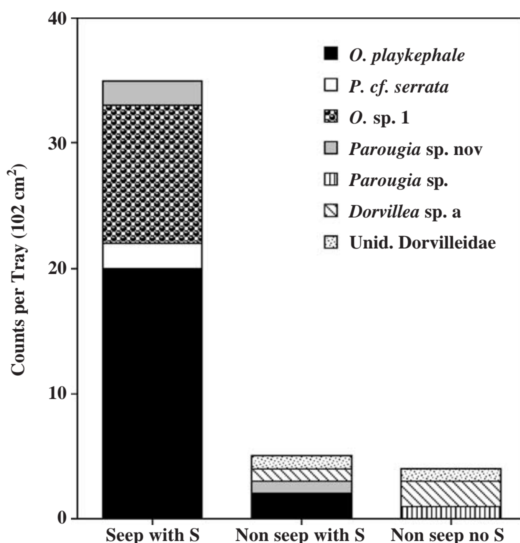

Fig. 6. Dorvilleid polychaete species composition in 3 colonization tray treatments after 6-month deployment at 525 m on the Eel R. margin. Counts are number per 102 cm^2 (9 cm deep) tray.

4. Discussion

4.1. Colonization rates

The trays employed in this study are a significant improvement over the raised, hydrodynamically biased

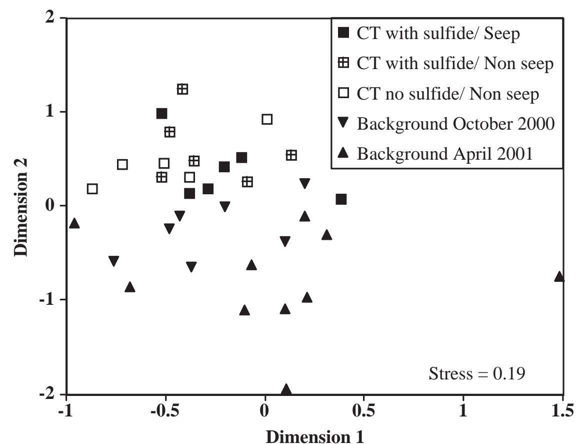

Fig. 7. Multidimensional scaling plot of macrofaunal communities (>300 μm) inhabiting natural sediments on the Eel R. margin (525 m) sampled in October 2000 and April 2001 (non-seep, clam bed and microbial mat) and 3 colonization tray treatments (as in Fig. 4).

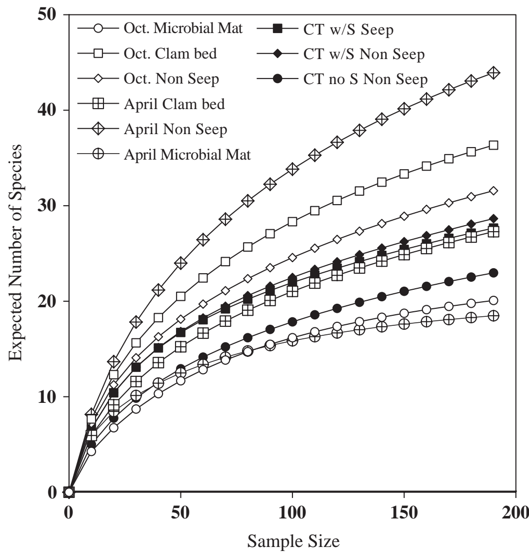

Fig. 8. Rarefaction plot illustrating diversity of macrofaunal communities (>300 μm) inhabiting natural sediments on the Eel R. margin (525 m) sampled in October 2000 and April 2001 (non-seep, clam bed and microbial mat) and 3 colonization tray treatments (as in Fig. 4). CT = colonization tray, w/ S = with sulfide added; no S = without sulfide added.

experiments deployed in early deep-sea colonization studies (reviewed in Smith, 1985; Smith and Hessler, 1987; Snelgrove et al., 1995). These early studies created a paradigm of low recruitment by ambient deep-sea fauna with opportunistic taxa dominating. The experiments presented here for a 500-m continental margin site revealed colonization rates consistent with other dynamic bathyal environments. Within 6 months, trays achieved about 50% of ambient October (start) densities, independent of treatment. The present findings of relatively rapid recovery of total non-seep densities is consistent with comparable experiments (using identical trays) by Snelgrove et al. (1992, 1994, 1996) at 900 m in the US Virgin Islands and by Levin and DiBacco (1995) at 580–600 m on Fieberling Guyot in the Eastern Pacific Ocean. Somewhat higher colonization rates (relative to ambient) but lower densities were attained on Fieberling Guyot where sediments are coarse-grained, flow rates and shear are high, and substrate mobility is frequent (Levin et al., 1994; Levin and DiBacco, 1995). With the exception of cumaceans, which were unusually well represented in colonization trays relative to background sediments (Table 1), we did not observe the density overshoots and opportunistic colonizers associated with organic enrichment treatments employed by Snelgrove et al. (1992, 1996) and Desbruyeres et al. (1980). In the present study most of the colonists were common in the background communities, consistent with an apparent difference between Atlantic and Pacific macrofaunal recolonization in the deep sea as noted by Levin and Gooday (2003).

The sulfide-laden trays failed to fully recover a seep macrofaunal community, though recruitment of characteristic seep infauna (e.g., *Calyptogena* sp., dorvilleid polychaetes, oligochaetes) was observed. The failure of sulfide to diffuse to the tray surface, and the lack of active pumping of ions (e.g., by clams) meant that trays did not fully mimic the geochemistry of microbial mat and clam bed sediments at the study site. Increases in methane seepage might have driven the density increases observed in seep and clam bed sediments between October 2000 and April 2001 (Levin et al., pers. obs), but these also were not simulated in the colonization trays.

4.2. Experimental design

The initial design of sulfide treatments was based on sulfide microprofiles made in Monterey Bay seeps by one of the authors (W.Z.), as the Eel R. seep sulfide concentrations were measured during the same cruise shortly after we initiated the tray experiments. This is a pitfall of limited (and expensive) ship time and work in inaccessible environments. Profiles in sulfide-addition trays made at the end of the tray deployment (Fig. 3) were similar to those in Eel R. clam bed sediments (Fig. 1). However, colonization trays did not simulate sulfide conditions in bacterial mat sediments, where sulfide is present all the way up to the sediment surface (Fig. 1). We postulate that sulfide presence at the surface would encourage bacterial mat taxa such as selected dorvilleid polychaetes to settle in greater abundance. Future deployments are planned to test this hypothesis.

4.3. Colonization patterns

Several taxa found to preferentially settle in or avoid sulfide-addition treatments exhibit preferences consistent with background distributions. *O. platykephale*, an abundant species in bacterial mats, settled only into sulfide/seep trays. Of two species exhibiting sulfide preference in the tray experiments, *Mediomastus* sp. was dominant in clam beds in Oct. 2000 and *Aphelochaeta* sp. H, was more abundant in clam beds during April 2001. Nerillid polychaetes, which settled only in sulfide-addition trays, are uncommon in Eel R. background sediments but are notably the only

dominant macrofauna in hypoxic Santa Barbara Basin sediments (580 m) covered with *Beggiatoa* mats (Müller et al., 2001; Levin and Bernhard, unpublished data). *N. cornuta*, which avoided our sulfide additions, was more abundant in non-seep sediments than seep sediments in October (Levin et al., 2003) and April (Table 2).

5. Conclusions and implications

Our original hypotheses about the influence of sulfide and seep habitat proximity could not be nullified for total macrofaunal abundances, but selected taxa clearly exhibited preferences for or against sulfide and seep proximity. As predicted, most of those taxa preferentially settling into sulfide addition trays or trays place within seep sites (dorvilleids, capitellids, oligochaetes) were species reported to have elevated concentrations in seep sediments (Sahling et al., 2002; Levin et al., 2000, 2003). Our hypothesis that sulfide additions might elevate species richness was supported, although the highest species numbers were found in trays with sulfide placed in seep patches.

The general patterns observed in colonization trays suggest that proximity of seep habitats had at least as great an influence on macrofaunal recruitment as tray hydrogen sulfide concentrations. Enhanced abundances of seep annelids in trays nestled within seep sediments may reflect proximity to source populations and limited dispersal ability of these taxa, or the influence of seep-associated geochemical cues emanating from sediments around the trays. Our colonization results support the paradigm that the deep sea consists of a mosaic of habitat patches with different successional dynamics (Snelgrove and Smith, 2002). Methane seeps are clearly a key contributor to this mosaic on continental margins.

Continental margin sediments are subject to increasing disturbance from trawlers, oil and gas exploitation and waste dumping (Smith et al., in press). Sablefish, also known as black cod (*Anaplopoma fimbria*), were abundant near the Eel. River seeps during October 2000. We observed trawlers fishing near our study area in October 2000 and found trawl tracks through our site in April 2001. Understanding the recovery potential and recovery rates of margin macrofauna in both seep and non-seep settings is essential for assessment of human disturbance, formulation of regulations to limit impacts, and the eventual conservation of both seep and non-seep environments. The chemistry and microbiology of seep sediments are likely to exert considerable influence over recruitment and population dynamics (Levin, 2005). Experiments offer a powerful approach to study this influence, but often require use of expensive deep-submergence vehicles and are constrained by availability of ship time at suitable intervals for deployment and recovery. For these reasons, linking the geochemistry of sediments to settlement and survival of margin assemblages in general, and seep communities in particular, remains a formidable challenge.

Acknowledgements

We thank the captain and crew of the RV Thompson and the ROV crews for the Jason I and Oceanic Explorer for invaluable assistance with the field program. Scientific assistance at sea was provided by A. Rathburn, J. Gieskes, K. Brown, C. Mahn, M. Tryon, J. Weinberger, B. Becker, L. Lovell, A. Cook, P. Yancy, K. Wilson, D. Morrow, K. Geigle, J. Holmer and D. Perkey. Assistance with identification of species was provided by L. Lovell and D. Cadien. Help in the laboratory with sorting was provided by Tina Reising. We thank 2 anonymous reviewers for comments on an earlier draft of this paper. Support was provided by NOAA NURC West Coat Grants UAF 00-0050, UAF 05-0141, and NSF Grant OCE 0425317. **[SS]**

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