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SPECIAL TOPIC

Diversity of bathyal macrofauna on the northeastern Pacific margin: the influence of methane seeps and oxygen minimum zones

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Abstract

The upper continental slope in the northeastern Pacific Ocean is intercepted by a deep oxygen minimum zone (OMZ; 650–1100 m) and punctuated by conduits of methane seepage. We examined the effects of these two dominant sources of heterogeneity on the density, composition and diversity of heterotrophic macrofauna off Hydrate Ridge, Oregon (OR; 800 m water depth), where the seeps co-occur within an OMZ, and off the Eel River, Northern California (CA; 500 m), where seeps are overlain by better oxygenated waters. We hypothesized that seeps (containing clam beds and microbial mats) should contribute a suite of distinct species to the regional margin species pool but that OMZ-associated hypoxia would dampen seep-related heterogeneity. Macrofaunal densities were highest (23,000–33,510 ind·m^{-2}) in the CA seep sediments and in the OR near-seep samples, intermediate in the OR seep, CA near seep and CA and OR 500-m margin sediments (10,054–19,777 ind·m^{-2}), and lowest in the CA and OR OMZ habitats at 800 m (4269–7847 ind·m^{-2}). Annelids constituted over 50% of the taxa in all but the CA clam bed and OR microbial mat sediments, where mollusks were abundant. Approximately 50% of seep species appeared to be habitat endemic; species present in microbial mats largely formed a subset of those present in the clam beds. Dorvilleid and ampharetid polychaetes were dominant in the seep sediments; non-seep margin sediments at 500 and 800 m were populated heavily by branchiate polychaetes including cossurids and paraonids. Alpha diversity (Es[20] calculated per core) was lowest and rank 1 dominance was highest in the CA and OR microbial mat habitats. Pooled analyses of Es[100] revealed highest species richness in the CA clam bed and near-seep habitats (30.3 and 29.6, respectively), and lowest species richness in the OR microbial mat and near-seep habitats (16.5 and 17.9, respectively). Non-seep sediments (500 and 800 m) off both CA and OR were more homogeneous (55–57% within-habitat similarity) than clam bed and microbial mat sediments (only 32–37% within-habitat similarity). CA sediment macrofauna generally exhibit higher alpha diversity, and as habitats are combined, a higher rate of increase in the slope of the species accumulation curves than do OR margin macrofauna. Methane seeps in the NE Pacific introduce significant heterogeneity that increases margin biodiversity at multiple spatial scales. However, our hypothesis that the OMZ would lessen the seep contributions to diversity was not supported. The better oxygenated CA seeps at 500 m shared more of the background margin fauna (at 500 m) than did the OR seeps at 800 m (with OMZ fauna at 800 m). Geographical differences in the fluxes of methane-rich fluids and the increased reliance on chemosynthetic food sources with increased depth could explain these results.

Problem

The bathyal sediments of continental margins (*i.e.* 200–3000 m), once considered homogeneous and rather food-poor, are now recognized as being highly heterogeneous as a result of tectonic, oceanographic and terrestrial forcing. This heterogeneity is manifested as topographic features, such as trenches, canyons, ridges, banks and gullies, geochemical features generated by subsurface fluid seepage or degradation of organic inputs from upwelling production or flood deposits, or hydrographic features such as oxygen minima, water masses with specific temperature and salinities, and boundary currents (*e.g.* Rathburn *et al.* 2009). Several of these features come together along much of the eastern Pacific margin to create environments characterized by oxygen depletion and methane seepage (e.g., Sellanes *et al.* 2010). Here we address their relative influences on community structure and biodiversity off Oregon (OR) and California (CA) bathyal macrofauna.

The role of habitat heterogeneity in generating and maintaining species diversity on margins is increasingly recognized. Initially, bathyal research focused on bathymetric patterns. Mid-slope depths were found to support maximal macrofaunal and megafaunal diversity (Rex 1983; Stuart *et al.* 2003), although the underlying mechanisms remain debated. Recent attention has shifted to the mechanisms underlying diversity, and their implications for conservation and management. Habitat and species diversity are recognized as key to many ecological functions and services on margins (Snelgrove *et al.* 2004; Danovaro *et al.* 2008). Margins support key fisheries and energy resources (Snelgrove *et al.* 2004), which are subject to increasing exploitation as similar resources are depleted in shallower waters (Glover & Smith 2003; Smith *et al.* 2008). The margins also play fundamental roles in global C cycling, accounting for significant C deposition and burial (Sarmiento & Gruber 2006).

The East Pacific margin, due to a combination of extensive plate subduction and long-term organic matter inputs from high surface production and terrestrial inputs, hosts extensive methane hydrate accumulations as indicated by bottom-simulating reflectors (BSRs) in sub-bottom acoustic profiles (*e.g.* Morales 2003). These are often manifested at the sediment surface as methane seeps (Hester & Brewer 2009). Methane seeps are especially common at water depths of 500–1000 m, where temperature and pressure combine to destabilize gas hydrates (Hester & Brewer 2009). In the Eastern Pacific the biota have been described for methane seeps off Chile (Sellanes *et al.* 2008), Peru (Olu *et al.* 1997), Costa Rica (Sahling *et al.* 2008), the San Clemente and Santa Monica Basins (Ussler *et al.* 2006), Monterey Bay (Barry *et al.* 1996), Northern California (Levin *et al.* 2003), Oregon (Juhl & Taghon 1993; Sahling *et al.* 2002), and Alaska (Wallman *et al.* 1997; Suess *et al.* 1999; Levin & Mendoza 2007). The study of methane seep biota, as with most deep-sea reducing ecosystems, has largely occurred in isolation from the surrounding, ambient deep sea. This is in part because these settings are distinct in substrate and fauna, and thus sampling methodologies often differ. However sediment-dwelling fauna are likely to encounter reducing microenvironments under many circumstances in the deep sea, for example at organic falls, in oxygen minima, or beneath organic depo-centers. Thus, we might expect that some fraction of seep infauna (particularly heterotrophic species) are not endemic to seep systems, but rather are associated with low-oxygen, sulfidic, or disturbed sediments. An extensive low-oxygen water mass blankets the bathyal East Pacific margin, creating approximately 360,000 km^2 of hypoxic sea floor (Helly & Levin 2004). This feature, termed an oxygen minimum zone (OMZ; oxygen concentrations $<0.5\ ml \cdot l^{-1}$) occurs at depths of ~650–1100 m off CA and OR (Helly & Levin 2004), and overlies several sites of methane seepage off OR.

This study addresses the influence of heterogeneity generated by the OMZ and methane seepage on the density, composition, and species diversity of northeast Pacific margin macrofauna. Both methane seeps and the oxygen minimum zones exhibit physiologically stressful conditions that are associated with reduced macrofaunal diversity (reviewed in Levin 2003, 2005), but they also support specialized, habitat-endemic species that may contribute to regional diversity on margins. We examined the role of geographical setting (California *versus* Oregon margins), sulfidic seep sub-habitats, and overlying low oxygen concentrations (the OMZ) in determining infaunal densities, composition and the alpha diversity of sediment-dwelling macrofauna (animals >0.3 mm). We compare macrofaunal assemblages in clam beds and microbial mat-covered seep habitats, in near-seep (edge) habitats, and in non-seep sediments at 500 and 800 m. The CA seeps studied are off the mouth of the Eel River at 500 to 525 m (Fig 1). They occur just above the OMZ in waters with higher dissolved oxygen concentrations (Fig. 2), although it is possible that the position of the upper OMZ boundary varies and may periodically include 500 m. On the OR margin we studied seeps at Southern Hydrate Ridge in 770 m water depth (Fig. 1), occurring well within the OMZ core (Fig.2). We hypothesized that (i) seeps are heterogeneous settings that contribute a suite of distinct species to the regional margin species pool and (ii) that OMZ-associated hypoxia acts to homogenize ecosystem composition such that (iii) seep-related heterogeneity should be manifested more strongly outside (at 500 m) than within the OMZ (at 800 m). These hypotheses would be supported if we find greater between-sample similarity within OMZs, more seep

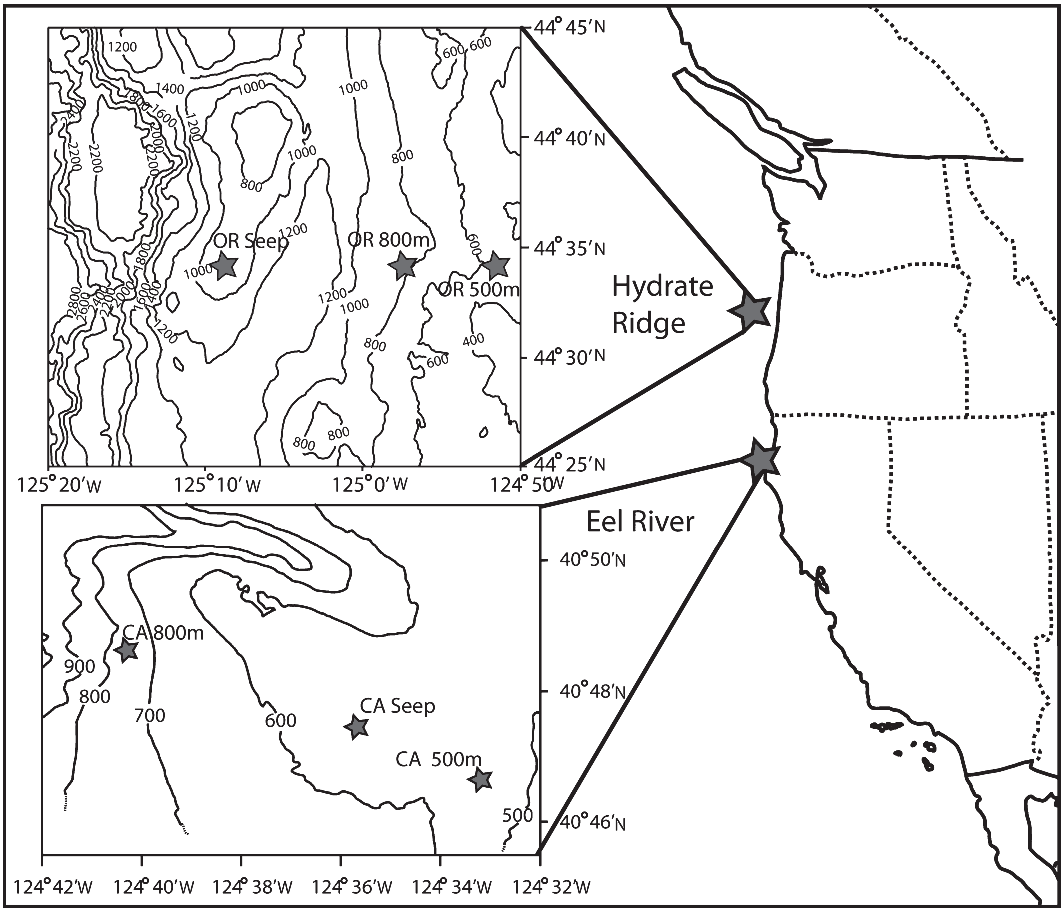

Fig. 1. Map showing location of the Eel River (CA) and Hydrate Ridge (OR) methane seeps and the 500-m and 800-m margin sampling sites.

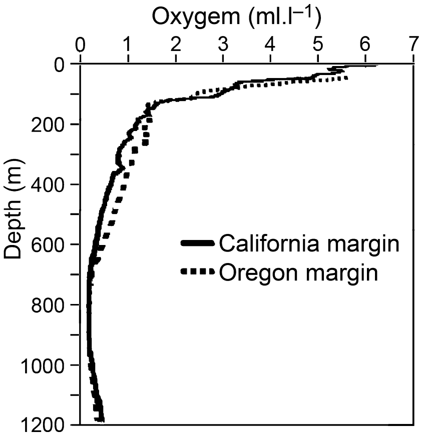

Fig. 2. Representative oxygen profiles on the OR and CA margins to 1200 m. Measurements are from a CTD oxygen sensor, calibrated with a Winkler apparatus. Note the oxygen minimum zone (<0.5 ml·l^{-1}) extends from about 600 to 1200 m.

endemics in more oxygenated sectors of the margin off CA, and more overlap between seep and non-seep faunas within the OMZ off Oregon.

Methods

Site characteristics

The research was conducted at seeps off Northern California (referred to as Eel River) and off Oregon (referred to as Hydrate Ridge), and on margin sediments within 22 nautical miles of the seeps at comparable depths (Fig. 1). The Eel River seep site, located from 500–525 m, is described in detail by Levin *et al.* (2000, 2003) and includes white microbial mats, vesicomyid clam beds and nearby (near-seep) sediments with scattered dead clamshells or carbonate rocks (Fig. 3). Bottom-water temperature at the seeps was 5–6 °C, and salinity was 33.2–33.6. The oxygen concentration in overlying water was variable, with recorded values as low as 0.24 ml·l^{-1} but usually >0.5 ml·l^{-1}, and up to 0.9 ml·l^{-1}. Thus we consider the Eel River seep site to be located above the OMZ, but the OMZ upper boundary may fluctuate such that the seep biota periodically experience lower oxygen associated with OMZ conditions. Anaerobic methane oxidation is a dominant process (Orphan *et al.* 2001). Oxygen penetration

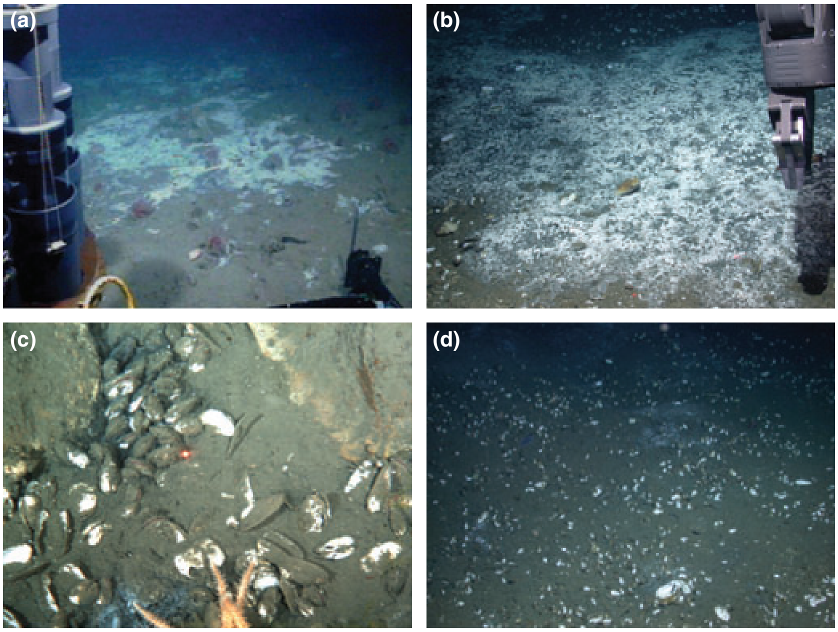

Fig. 3. Images of microbial mat-covered seep sediments off CA (a) and OR (b), and clam bed habitat off CA (c) and OR (d).

into Eel River sediments was 4, 3 and 0 mm in near-seep, clam bed and bacterial mat sediments, respectively, with porewater hydrogen sulfide levels of ~ 0.2 mM, 2 mM and 14–20 mM in the upper 5 cm, respectively (Levin *et al.* 2003). Clam bed sediments have 1% organic carbon, a C:N ratio of 16.8, a high proportion of sand (69%) and abundant authigenic carbonates (Table 1).

Hydrate Ridge is one of several ridges along an accretionary prism formed where the Juan de Fuca plate is being subducted under the North American Plate. The ridge, associated with gas hydrate formation, is 25 km long and about 15 miles wide. Extensive methane seepage occurs in three regions on Hydrate Ridge; sampling for this study focused on the southern summit (770 m; hereafter Hydrate Ridge). Key seep habitats at Hydrate Ridge include microbial mats, *Calyptogena pacifica* and *Calyptogena kilmeri* beds (Fig. 3) and surrounding *Acharax* beds (Sahling *et al.* 2002). These are associated with decreasing methane fluxes and porewater H_2S concentrations, respectively. Anaerobic methane oxidation rates are high (Boetius *et al.* 2000; Treude *et al.* 2003). Within the microbial mats, H_2S at Hydrate Ridge can reach 25 mM or more and is present at the sediment–water interface. Clam bed H_2S levels can reach 5–8 mM (Sahling *et al.* 2002; W. Ziebis personal communication) and are higher than at Eel River. Dissolved oxygen concentrations in water 5 m above the sea floor during our 2006 cruises was 0.2–0.3 $\text{ml}\cdot\text{l}^{-1}$, but Sahling *et al.* (2002) report oxygen at Hydrate Ridge as 0.40–0.47 $\text{ml}\cdot\text{l}^{-1}$. Bottom-water temperature at 770 m was about 4 °C. Organic C contents of surficial microbial mats can be as high as 4.4%; values are lower in clam bed (1.5%) and reference sediments (2%). However, % N content is higher in clam bed and reference sediments than in microbial mats and C:N ratios are lower (3.8–9.3 *versus* 42.7 in mat sediments) (Valentine *et al.* 2005; Table 1).

Non-seep margin/OMZ sediments were coarser grained and had lower organic C contents at 500 than 800 m (in the OMZ) off CA and OR (Table 1). For a fixed water depth, sediment organic content was higher and the C:N ratio was lower off OR than off CA (Table 1).

Sampling

Sampling of margin sediments off seeps was conducted from the RV *Atlantis* on the OR and CA margins in July 2006 by multicorer at depths of 500 and 800 m. We examined a minimum of three tube cores from each of three multicore drops at each depth station (Table 1). Cores were 9.6 cm internal diameter × 10 cm deep. As discussed above, the 800-m samples on the CA and OR margin were within the OMZ and are referred to as OMZ samples.

Seep samples were collected by tube core (7 and 8.3 cm diameter × 10 cm depth) at Hydrate Ridge, OR (770 m) during August 2005 with the ROV *Tiburon*, aboard the RV *Western Flyer*. Samples were taken at Eel River seeps off CA (500–525 m), and Hydrate Ridge seeps in July and October 2006 with the submersible *ALVIN*, aboard the RV *Atlantis*. Sediments were sampled by tube cores of 6.4 or 8.3 cm diameter × 10 cm depth). The ROV- and submersible-collected sediments were classified

Table 1. Study site locations, sediment properties and numbers and sizes of core samples.

				sediment properties [mean (SE)]					number of macrobenthos samples			
	depth (m)	latitude °N	longitude °W	n	%C	%N	C:N	% sand	multicore (78.3 cm^2)	large tube cores (54.1 cm^2)	small tube cores (38.5 cm^2)	box core (49 cm^2)
California												
CA margin	500	40.8	124.5	3	0.68 (0.05)	0.079 (0.004)	8.5	34.7 (2.1)	12	0	0	0
CA clam bed (seep)	500–525	40° 47.19	124° 35.7	1	0.97	0.1	16.8	69.0	0	12	7	1
CA microbial mat (seep)	500–525	40° 47.19	124° 35.7						0	5	2	1
CA near seep	500	40° 47.19	124° 35.7						0	7	0	1
CA OMZ	800	40.8	124.7	2	1.07 (0.02)	0.122 (0.005)	8.7	11.0	9	0	0	0
Oregon												
OR margin	500	44.6	124.9	1	0.9	0.1	7.8	63.0	9	0	0	0
OR clam bed (seep)	770	44° 34.22	125° 8.80	1	1.5	0.2	8.9		0	16	7	0
OR microbial mat (seep)	770	44° 34.22	125° 8.80	1	4.4	0.1	42.7		0	11	4	0
OR near seep	770	44° 34.22	125° 8.80						0	0	2	0
OR OMZ	800	44.6	125.0	3	2.14 (0.09)	0.279 (0.009)	7.7 (0.09)	13.2	9	0	0	0

Sediment data sources: CA clam bed (Levin *et al.* 2000), OR clam bed, microbial mat, near seep (Valentine *et al.* 2005), CA and OR slope at 500 m, 800 m – new data.

as 'clam bed' (beds of *Calyptogena pacifica at* Eel River and *C. pacifica* and *Calyptogena kilmeri* at Hydrate Ridge), 'microbial mat' (covered with white, filamentous sulfide-oxidizing microbes), or 'near-seep' sediments without obvious manifestations of seep organisms on the sediment surface located within a 300 × 500 m area containing the Eel River seep (these were referred to as non seep in Levin *et al.* 2003). To obtain more complete species lists, we have also included data from 8.3-cm-diameter tube cores taken in clam bed, microbial mat and near-seep sediments at the same Eel River sites in October 2000 with the ROV *Jason II* (15 cores) and in April 2001 with the ROV *Oceanic Explorer* (10 cores). The macrofaunal data for the 2000 and 2001 samples were published in Levin *et al.* (2006). Sample numbers for each location and habitat type are given in Table 1. Samples were fractioned vertically at sea. The upper 5 cm were preserved unsieved, the 5–10-cm fraction was sieved on a 0.3-mm mesh for multicore samples and all other cores were preserved vertically fractioned but unsieved to 10 cm. All fractions were preserved in 8% buffered formalin and re-sieved in the laboratory on a 0.3-mm mesh. Invertebrates in retained sediments were sorted at 12× magnification and identified to the species level (Polychaeta) or lowest possible taxon (usually order or family). All specimens were assigned to putative species (unnamed) for calculation of diversity.

Statistical tests

Counts of density, species richness, and diversity indices were tested for normality (Shapiro–Wilk test) and log-transformed to achieve normality when possible. Comparisons across depths and habitats were made with univariate analyses (One- and Two-way ANOVA, Tukey's HSD test *a posteriori*) or when normality could not be achieved, with Kruskal–Wallis tests, using JMP 6.0.3. Multivariate analyses of community structure (Bray–Curtis Similarity measures, MDS), within-habitat (SIMPER) and between-habitat differences (ANOSIM), diversity indices (S, d, J' H' and $ES_{(20)}$, $ES_{(100)}$) and rarefaction curves were generated with PRIMER (6.0) software.

To assess the additive contributions of different habitats and the relationship between species richness and habitat number, species presence/absence data were subjected to an expanded rarefaction analysis. Rarefaction curves (number of species per number of individuals sampled) were generated for random combinations of data from every possible combination of 1, 2, 3, 4, or 5 habitats. This was done for CA and OR samples separately, and for combined data. When habitats were combined, each habitat was randomly subsampled to the level of the lowest number of samples within an individual habitat to ensure equal replication during the generation of a rarefaction curve. The curves were log-transformed to linearize them and the slopes of the resulting rarefaction curves were determined for each habitat combination and plotted as a function of habitat number. A complete treatment of this method is described in Cordes *et al.* (2010).

Results

Densities

A total of five habitats (upper margin at 500 m, OMZ at 800 m, clam bed, microbial mat, and near-seep) were studied in each of two regions (CA and OR). Macrofaunal densities in these settings ranged from 4,270 to 33,510 ind·m^{-2} (Table 2). Densities were highest (23,000–33,510 ind·m^{-2}) in the CA clam bed and microbial mat habitats and the OR near-seep samples, intermediate in the OR clam beds and microbial mats, CA near-seep and CA and OR upper margin sediments (10,054–19,777 ind·m^{-2}) and were lowest in the two OMZ habitats at 800 m (4,269–7,847 ind·m^{-2}) (One-way ANOVA, $F_{9,114} = 8.394$; $P < 0.0001$). However, only the CA clam bed and the OR margin OMZ sites exhibited significant density differences from the other sites (Tukey's HSD). Seep macrofaunal densities (in clam beds and microbial mats), were about twice those in non-seep sediments at comparable depths off CA (500 m) and four times those of non-seep OMZ sediments off OR (800 m) (Table 2). Within each region, macrofaunal densities in clam beds did not differ from microbial mat sediments (Table 2; ANOVA, CA – $P = 0.139$, OR – $P = 0.409$). Near-seep and off-seep densities were comparable at 500 m off CA (about 13,900 ind·m^{-2}), but at 800 m off OR, limited sampling suggests macrofaunal densities in near-seep sediments (29,608 ind·m^{-2}) were seven times higher than in the OMZ (4,269 ind·m^{-2}). Macrofaunal densities exhibit significant location effects (CA *versus* OR) and habitat effects (Seep *versus* 500 m margin *versus* 800 m OMZ) (Two-Way ANOVA; $F_{5,114} = 11.94$, $P < 0.001$) with no habitat–location interaction.

Composition

Annelids made up over 50% of the taxa in all but two of the habitat/location combinations examined (Table 2). Annelids were numerically dominant (75–83%) in the CA and OR OMZ, in the CA microbial mat, and in the OR clam bed habitats. They constituted 69–70% of the fauna at the CA and OR near-seep and 500-m margin habitats, but only 59–43% of the CA clam bed and OR microbial fauna. Mollusks were relatively poorly represented (2–7%

Table 2. Summary statistics for macrobenthos from margin, oxygen minimum zone (OMZ), clam bed, microbial mat and near-seep habitats off California and Oregon.

	CA margin	CA clam bed	CA microbial mat	CA near seep	CA OMZ	OR margin	OR clam bed	OR microbial mat	OR near seep	OR OMZ
depth (m)	500	500	500	500	800	500	770	770	770	800
surface area sampled (cm^2)	869	935	387	428	652	652	1103	731	67.8	652
density ind.m^{-2}	13,993	33,510	23,326	13,860	7847	10,054	19,777	16,407	29,607	4,269
SE	1170	5106	7053	1361	3022	1157	3119	2522	10,900	216
% Annelids	60.6	55.8	78.8	55.5	82.8	67.5	75.9	43.5	71.6	76.2
% Molluscs	2.7	22.7	6.7	6.8	1.9	21.7	19.9	52.5	24.1	6.1
% Crustaceans	35.4	11.6	7.7	25.2	10.1	6.9	3.3	3.2	3.9	15.1
% Echinoderms	0.3	0.6	0.2	0.5	1.7	2.4	0.2	0.1	0	0.7
% Nemerteans	0.7	8	5.7	11.2	2.7	0.8	0.7	0.7	0.4	1.4
% Dorvilleids	0.6	28.8	67.4	3.3	1.7	1	20.5	8.4	32.6	7.5
No. species	42	88	49	60	29	48	55	38	23	29
Percent of spp. unique to the habitat (within region)	19	22	8	13	25	46	24	13	4	17

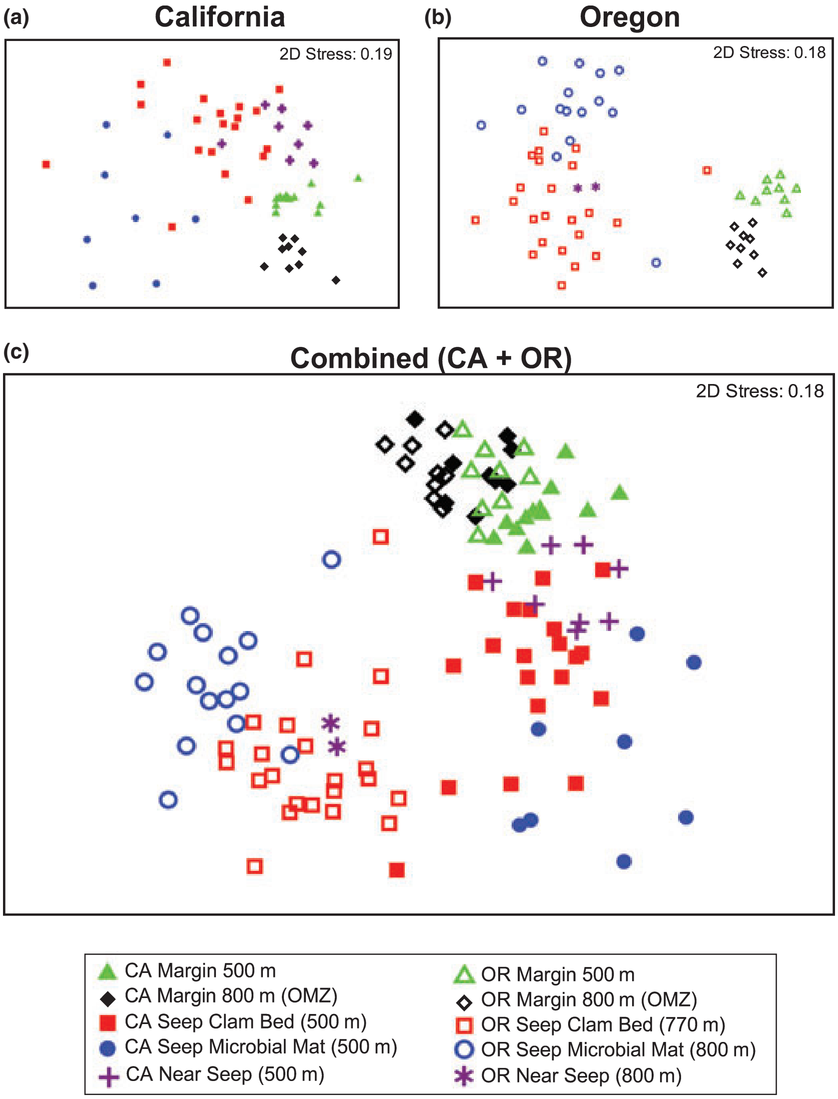

Fig. 4. MDS Plots of macrofauna (500–800 m) from (a) the Oregon margin, (b) the California margin at 500 m, and (c) the Oregon and California margins.

of individuals) at all of the CA habitats except the clam bed, and in the OR OMZ. However, they made up 21–53% of the fauna in the remaining OR habitats and in the CA clam bed sediments. Arthropods (mainly amphipods and cumaceans) exhibited the reverse pattern from mollusks. They were less common (3–5% of individuals) at all the OR sites but the OMZ (16%), and better represented in the CA habitats [6–10% of individuals at all but the CA margin (28%)]. Nemerteans formed 6% to 14% of the fauna in the three CA seep habitats, 3% in the CA and OR OMZ but were <1% of the fauna in the other settings. Echinoderms were <1% of the fauna throughout the study region, except in the CA OMZ and at 500 m on the OR margin (2–3%).

Of the 148 taxa encountered, 27 attained densities of 1000 ind·m^{-2} or higher in one or more settings. There were clear differences in dominant taxa between regions and habitats. Two ampharetid polychaetes attained high densities in OR seep habitats, and six dorvilleid polychaete species attained high densities in CA and OR seep sediments. The non-seep margin sediments at 500 and 800 m were populated heavily by cossurid, nephtyid and paraonid polychaetes, gammarid amphipods and cumaceans off CA, but capitellid, cirratulid, and paraonid polychaetes and bivalves off OR. Among the top 10 ranked species in the OMZ (800 m), eight of 10 were polychaetes with branchial and tentaculate structures (cossurids, cirratulids, paraonids, spionids and ampharetids)

Table 3. Dissimilarity between habitats (above diagonal), within-habitat similarity (diagonal, bold), and ANOSIM probabilities (below diagonal).

(a) California (counts, 4th root-transformed)						
R = 0.575		margin	clam bed	microbial mat	near seep	OMZ
P = 0.001	margin (500 m)	**56.75**	74.28	85.69	67.97	60.19
	clam bed	0.001	**32.4**	79.08	70.72	81.99
	microbial mat	0.001	0.002	**37.28**	82.15	88.61
	near seep	0.001	0.019	0.001	**45.19**	78.09
	OMZ (800 m)	0.001	0.001	0.001	0.001	**57.32**
(b) Oregon (counts, 4th root-transformed)						
R = 0.669		margin	clam bed	microbial mat	near seep	OMZ
P = 0.001	margin (500 m)	**57.21**	92.05	93.74	91.15	61.87
	clam bed	0.001	**32.74**	74.54	62.62	89.91
	microbial mat	0.001	0.001	**36.48**	69.61	91.6
	near seep	0.018	0.853	0.018	**51.8**	83.6
	OMZ (800 m)	0.001	0.001	0.001	0.018	**54.61**
(c) California *versus* Oregon – within-habitat comparisons of dissimilarity						
CA *versus* OR						
	margin (500 m)	64.17				
	clam bed	86.03				
	microbial mat	94.59				
	near seep	86.64				
	OMZ (800 m)	59.54				

off CA; four of the top 10 species belonged to these groups off OR (Appendix S1).

Assemblage similarities

In both regions considerable species overlap was observed between clam bed, microbial mat habitats, and near-seep habitats (Appendix S2). For example, clam beds contained 84% of macrofaunal species present in the local microbial mat off CA and 76% off OR. Clam beds contained 80% of species in the near-seep habitat of CA and 96% off OR. Microbial mats supported a subset of the clam bed species off CA and OR (only 46% and 51%, respectively). Notably, off CA and OR, the near-seep habitat shared more species with the clam bed than with either of the non-seep habitats (Appendix S2), suggesting there was seepage influence or a proximity effect of some sort.

The above result is also evident in multivariate representation of the data (Fig. 4). OR non-seep habitats separate very clearly from seep habitats in multidimensional scaling plots (Fig. 4b,c), whereas the CA non-seep habitats more closely resemble the CA near-seep and clam bed settings (Fig. 4a,c). Multivariate comparisons of CA *versus* OR macrofauna (Table 3; SIMPER) reveal much greater similarity of non-seep (500 and 800 m) assem-

Table 4. Diversity indices for macrobenthos in margin, OMZ and seep habitats off California and Oregon.

	California margin					Oregon margin				
habitat	margin	OMZ	near-seep	clam bed	microbial mat	margin	OMZ	near seep	clam bed	microbial mat
water depth	500 m	800 m	500 m	500 m	500 m	500 m	800 m	770 m	770 m	770 m
cores averaged										
mean ES(20)	8.3	8.4	9.9	8.5	7	10.2	9	8.9	7.5	5.4
SE	0.3	0.4	0.4	0.3	0.7	0.4	0.5	1.1	0.3	0.3
cores pooled										
S	42	36	60	88	49	48	34	23	55	38
ES(100)	19.12	22.40	29.56	30.28	24.91	26.74	22.91	17.92	24.74	16.53
$H'(\log_e)$	2.74	2.71	3.19	3.31	2.78	3.03	2.84	2.65	2.92	2.32
J′	0.73	0.76	0.78	0.74	0.72	0.78	0.81	0.84	0.73	0.64
R1D	0.119	0.285	0.168	0.123	0.328	0.165	0.230	0.188	0.238	0.330

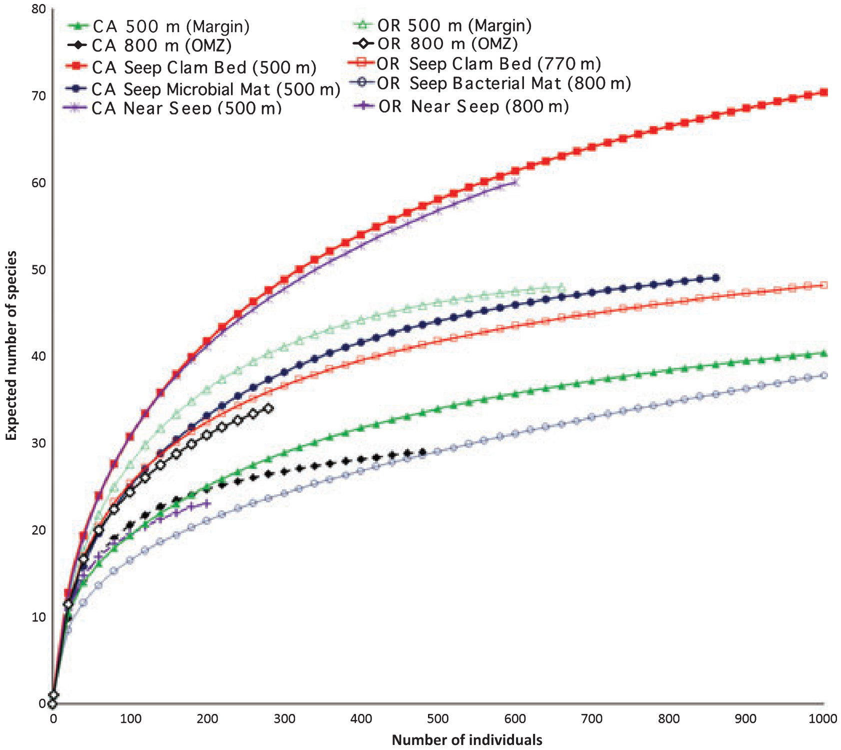

Fig. 5. Rarefaction curves showing richness of macrofauna from 500 m, 800 m (OMZ), seep (clam bed, microbial mat), and near-seep habitats on the California and Oregon margins.

blages (60–64% dissimilarity) than seep assemblages (86–95% dissimilarity). Significant geographic differences were evident between CA and OR macrofaunal assemblages for all habitat types (ANOSIM, all $P = 0.001$, except near-seep sediments where significance was weaker, $P = 0.022$).

Significant assemblage differences (ANOSIM, $P = 0.001$) were found among all habitats within a region, except between OR near-seep sediments and clam beds (Table 3a,b). Both CA and OR exhibited greater within-habitat similarity (*i.e.* homogeneity) within non-seep margin sediments at 500 m and OMZ sediments at 800 m (55–57% similarity) than in clam bed or microbial mat sediments (32–37% similarity) (Table 3a,b).

We did not find reduced between-habitat heterogeneity within the OMZ as predicted. In fact we saw the opposite, with more dissimilarity between OMZ and seep habitats off OR (84–92%) than off CA (78–89%) (Table 3a,b). We note with interest that the CA OMZ site at 800 m shared more species with the CA clam bed at 500 m (64%) than with any of the other CA habitats (Table 3), whereas the OR OMZ site at 800 m shared only 35% of the OR clam bed species. The geochemistry of OMZ and clam bed sediments is expected to be more similar in CA seep sediments, where the sulfide levels are lower than at the OR seeps.

Species richness and dominance

Different-sized cores were used for sampling seep, margin and OMZ sediments (Table 1), thus species richness comparisons are limited primarily to rarefaction analyses, which eliminate effects of sample size. Average estimated species richness per core (Es[20] calculated on a per core basis) was lowest in the CA and OR microbial mat habitats (7.0 and 5.4 species, respectively) and highest in the CA near-seep and OR 500 m margin habitats (9.9 and 10.2 species, respectively) (Kruskal–Wallis: $\chi^2_9 = 53.55$; $P < 0.0001$). The ES[20] of the OR microbial mat was significantly lower than in all other habitats (Tukey's HSD, all between 0.03 and <0.0001) except the CA microbial mat. The ES[20] of the CA microbial mats and OR clam bed were only significantly lower than in the OR margin at 500 m (both $P < 0.0001$) and CA near-seep habitats (both $P = 0.002$). A Two-way ANOVA ($F_{3,114} = 14.95$; $P < 0.0001$) yielded no effect of geographic location (CA *versus* OR) on ES[20] but did show a significant difference between seep and non-seep habitats ($P < 0.0001$) and a significant interaction between location and habitat ($P < 0.0001$). Notably, habitat non-seep diversity (ES[20]) was higher off OR than CA, whereas seep diversity was higher off CA than OR (Table 4).

Rarefaction analyses conducted per core yield somewhat different diversity rankings than the pooled analyses (Fig. 5) because of habitat differences in between-core heterogeneity (Table 3). Es[100] calculated by pooling all cores from a given habitat revealed lowest species richness in the OR microbial mat and near-seep habitats (16.5 and 17.9, respectively) and highest species richness in the CA clam bed and near-seep habitats (30.3 and 29.6, respectively). H' ($\log_e$) yielded essentially the same patterns (Table 4). Evenness (J') was notably lower in the OR microbial mat (0.64) and highest in the OR near-seep sediments (0.84) but did not vary greatly among the other settings. Rank 1 dominance, an inverse indicator of assemblage evenness, was very high and similar (0.33) in the CA and OR microbial mats, intermediate in the CA and OR OMZ (0.29 and 0.23, respectively) and lowest (0.12–0.19) in the CA and OR 500 m margin and near-seep sediments, and in the CA clam bed (Table 4).

Accumulation of species with habitat number

The analysis of habitat contribution to regional diversity (Fig. 6) plots initial slopes of accumulation curves for different numbers of habitats. The slopes of the curves are greater in general for the CA samples as opposed to the OR samples, suggesting a lower average pairwise similarity of CA communities. There is also a slightly lower habitat effect on beta diversity in the OR habitats. In other words, as habitats are combined in the analysis, the rate of increase in the slope of the accumulation curves is significantly higher in the CA habitats ($t = 2.34$, $P = 0.023$). This follows the overall greater rate of increase in the rarefaction analysis for CA than OR habitats (Fig. 5), but is contrary to the higher degree of dissimilarity among the OR habitats (Table 3). Even though there is greater dissimilarity among the habitat-specific species in OR, the decreased richness of OR keeps the total rate of increase limited and less than CA (Table 3). In the two-habitat comparison, the slope of the accumulation curves will approximate the dissimilarity between the habitats. Off CA the most dissimilar habitats (highest habitat heterogeneity) were the clam bed paired with near-seep or slope habitats. In OR, the highest species heterogeneity was found between the near-seep and slope followed by the clam bed and OMZ.

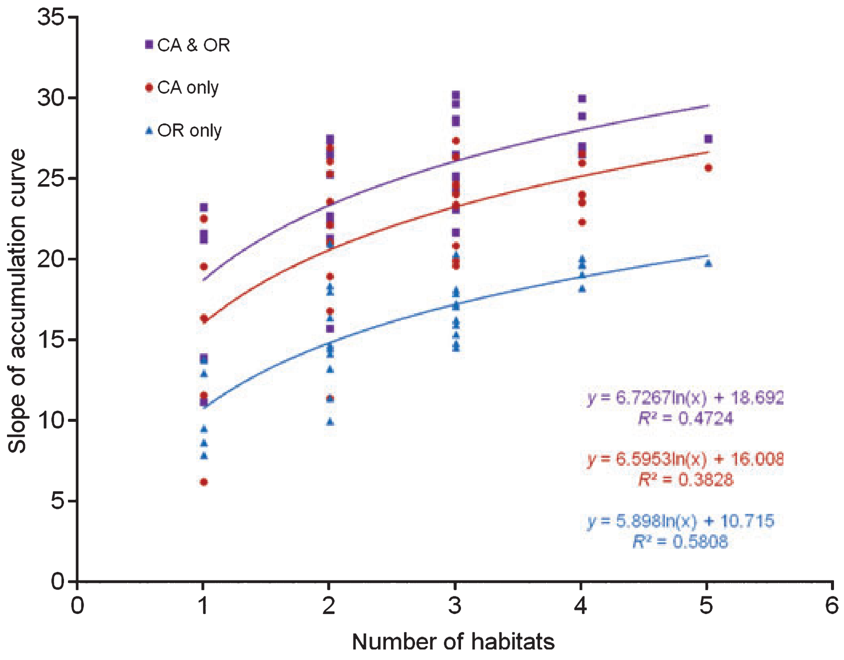

Fig. 6. Habitat contribution to diversity revealed from plots of the slopes of species accumulation curves for increasing numbers of habitats drawn from the following: clam bed, microbial mat, near seep, 500-m slope and 800-m slope (OMZ). Curves are shown for Oregon and California macrofauna separately and combined.

Habitat endemism

Contrary to our expectations, we did not see a higher percent of unique taxa in CA (non-OMZ) than OR (OMZ) seeps. In fact we saw a comparable fraction of habitat specialists (species found in one habitat only) in the CA clam bed (22%) and OR clam bed (24%), but surprisingly few habitat specialists in the CA and OR microbial mats (8% and 13%, respectively). This analysis is biased because we have separated the clam bed and microbial mat habitats. In the CA samples, 59% of the species present in seep and near-seep sediments were not recorded in CA non-seep sediments. In the OR samples, 52% of the seep and near-seep species were not found at the OR non-seep stations (Appendix S1). Thus, just over half of the seep macrofauna fauna appear to be seep specialists. Taxa specific to the seeps included dorvilleid, ampharetid, syllid, other polychaete and gastropod species (Appendix S1).

Discussion

Methane seep contributions to margin heterogeneity

Methane seepage contributes to regional margin biodiversity on several scales. At the largest scale, the seep clam bed and microbial mat habitats contributed 40 macrofaunal species (27% of all species collected) that were not present in the near-seep or non-seep margin settings (Appendix S1). An additional 22 seep species were also present in near- seep habitats that were not present at 500 or 800 m off seeps, suggesting that overall, ~42% of the regional species collected in this study may be associated with seepage activity. Nearly all of the macrofaunal species sampled are heterotrophic. Their association with seep habitats may be linked to an elevated supply of free-living microbial food sources or to tolerance to high sulfide concentrations (which range from 1 to >20 mM), facilitating their access to chemoautotrophic production.

We observed geographic heterogeneity between seep locations. The CA and OR sites are separated by 425 km but also by approximately 250 m of water depth. The CA seeps were more diverse than the OR seeps (Fig. 5) and the elevated species richness and diversity of the CA seeps led to greater species accumulation rates in the CA region (Fig. 6), emphasizing the significance of these habitat types to regional diversity. Two sources of stress could lower diversity at the OR seeps relative to CA seep: (i) OMZ-induced effects of lower bottom-water oxygen concentrations or (ii) higher porewater sulfide concentrations resulting from greater rates of fluid flux (Tryon & Brown 2001; Sahling *et al.* 2002; Levin *et al.* 2003) and higher rates of sulfate reduction (Treude *et al.* 2003; Valentine *et al.* 2005) off OR than CA. The greater depth of the OR than CA seeps would not be expected to affect the observed trend alone, as diversity usually increases rather than decreases with increasing water depth along the upper continental slope when OMZs are not involved (Rex 1983). Observation of an opposite trend for diversity at the 500- and 800-m non-seep stations (higher diversity off OR than CA), suggests that larger-scale regional hydrographic processes do not drive the seep diversity patterns.

Varying fluid flux regimes and associated microbial activity creates distinct clam bed and microbial mat habitats at Hydrate Ridge (OR) (Tryon & Brown 2001; Sahling *et al.* 2002) and Eel River (CA) seep sites (Levin *et al.* 2003). These occur in patches on the sea floor at scales of meters to tens of meters (Sahling *et al.* 2002; Levin *et al.* 2003; unpublished observation). Similar clam bed and microbial mat patch types and scales have been observed at seeps in the Northeast Atlantic and Mediterranean Sea (Vanreusel *et al.* 2009). Sediments beneath microbial mats typically have 5–10× higher porewater sulfide concentrations and exhibit much lower macrofaunal diversity (higher dominance) than sediments in clam beds (Sahling *et al.* 2002; Levin *et al.* 2003). The microbial mat fauna reported here appears largely to be a stress-tolerant subset of the clam bed fauna, with 76% (OR) to 84% (CA) of the mat species present in the clam bed sediments. In CA the microbial mat fauna consists largely of dorvilleid polychaete species. Off OR they are mainly gastropods and ampharetid polychaetes. Only a few species were found exclusively in microbial mat sediments; these included two gastropod species and two dorvilleid polychaete species.

Macrofauna and meiofauna characteristically exhibit distinct assemblages associated with ‘habitat’ types within seep settings (Cordes *et al.* 2010). Habitats are usually recognized and described by characteristic surface biota, including microbial mats, pogonophoran (frenulate) beds, clam beds, bathymodiolin mussels and lamellibrachid and escarpid tubeworms. Van Gaever *et al.* (2006, 2010) have documented distinct nematode genera in microbial mats, pogonophoran (frenulate siboglinid) fields, and reduced black sediment habitats at the Håkon Mosby mud volcano, in Nyegga pockmarks and in the Gulf of Guinea. Metazoan meiofauna and foraminifera in the Gulf of Mexico and Blake Ridge differ in mats of large sulfur bacteria and at the mussel bed periphery, but not among different types of microbial mats (Robinson *et al.* 2004). Macrofaunal assemblage composition varies over tens of meters in the West African pockmarks (Olu-Le Roy *et al.* 2007) and in the Mediterranean Sea (Vanreusel *et al.* 2009). Macrofaunal assemblages vary on the Aleutian margin between clam beds and pogonophoran fields and at the Florida escarpment between microbial mats and pogonophoran fields (Levin & Mendoza 2007). Tube worm assemblages house distinct macrofaunal communities in the Gulf of Mexico that vary within a given site and in a predictable successional sequence (Bergquist *et al.* 2003; Cordes *et al.* 2005), although the species composition of the initial stages of these communities may vary among sites (Cordes *et al.* 2006). Mussel communities are also distinct from the surrounding benthos on the Blake Ridge (Turnipseed *et al.* 2003) and may represent early successional stages in the Gulf of Mexico (Bergquist *et al.* 2005). In many of the above examples involving very small (<1 cm) taxa, single species may account for a disproportionately large fraction of the fauna, and these species vary among habitats (Robinson *et al.* 2004; Van Gaever *et al.* 2006; Levin & Mendoza 2007). It appears that in all regions where methane seeps have been studied they contribute to enhanced regional diversity through within-seep habitat heterogeneity (also see Cordes *et al.* 2010).

Seep assemblages also exhibit high levels of heterogeneity at the scale of tens of centimeters to a meter between tube cores. Within-habitat similarity among core samples for seep habitats within a region (SIMPER 32–37%) was much lower than that of the 500-m or 800-m margin sites (55–57%) (Table 3). High between-core heterogeneity was noted by Robinson *et al.* (2004) for meiofauna in the Gulf of Mexico. Considerable small-scale heterogeneity of seep assemblages may be the result of fine-scale (cm) spatial variation in geochemistry and microbial activity, as well as substrate variation associated with precipitation of carbonate pebbles, disturbance from active venting, or intense predator activity. Small-scale (mm to cm) variation in faunal distributions occurs both horizontally and vertically within the sediments (Levin *et al.* 2003) associated with vertical gradients in sulfide driven by seepage rates and archaeal and bacterial activity (Treude *et al.* 2003; Ziebis & Haese 2005).

Oxygen minimum zone effects on heterogeneity and diversity

In contrast to the strong spatial heterogeneity associated with seepage, a known effect of well-developed OMZs is to homogenize assemblages (Levin *et al.* 2000). Thus we hypothesized that OMZ-associated hypoxia should act to homogenize ecosystem composition on the CA and OR margins and thus limit the manifestation of seep heterogeneity at 800 m off OR. Our hypothesis that seeps in more oxygenated realms (in this case at 500 m) would have a more distinct fauna than seeps in the OMZ, was not supported. A comparison of species lists in seep habitats with those in non-seep sediments at comparable depths yielded 52–69% overlap off CA, where the seeps were exposed to higher oxygen concentrations, but only 32–35% overlap off OR, where seeps occurred at a deeper depth and within the OMZ (Appendix S2). The CA seeps introduced less species heterogeneity.

The overall rate of species accumulation was reduced in OR habitats (Fig. 6), but the lower basal habitat diversity off OR saw the greatest increase of diversity from the addition of increasing habitats. This was true even though the sum total richness was lower. Almost certainly the stronger fluid fluxes and higher sulfide levels at the Hydrate Ridge (OR) seeps made these sediments more dissimilar to the comparison margin sites.

Seeps and vents are known to support increasingly specialized faunas with increasing water depth (Sahling *et al.* 2003; Tarasov *et al.* 2005). We observed a similar trend when they interacted with OMZs. We speculate that the seep-associated inputs of microbial food may have had a larger effect on the macrofauna at 770 m than at 500 m, as the amount of particle rain from surface waters should be higher at shallower depths. Also, the CA site receives episodic organic inputs from massive flooding of the Eel River which send wood debris and fine-grained sediments to the upper slope, sometimes via small channels (Syvitski & Morehead 1999).

Shallow vent and seep waters support few symbiont-bearing species; a greater food supply in shallow water is the common explanation. In our study diversification at depth may result from increased niche space formed by a combination of unique food supplies (*i.e.* microbial groups) and refuge from ‘inhospitable’ environments by physiological adaptations (dorvilleids) or morphological adaptations (*e.g.* expanded branchial structures in ampharetids, cossurids, spionids). Another possible influence is the stronger water motion (and coarser particle sizes) associated with 500 m relative to 800 m on the CA and OR margins. It may be that relatively strong currents and substrate disturbance counters the development of habitat-specific assemblages, as suggested for shelf seeps off Northern California (Levin *et al.* 2000). Divers in *ALVIN* at the CA seeps off Eel River typically encountered strong flows of 25 $\text{cm}\cdot\text{s}^{-1}$ or more; these seemed to vary over tidal cycles and may be associated with regional internal tides, jets or streams.

While this study has focused on small invertebrates, larger symbiont-bearing megafauna may be more affected by low bottom-water oxygen concentrations. We speculate that the vestimentiferan tubeworms and mussels may be absent from many of the shallower East Pacific margin seeps off CA and OR because oxygen concentrations are insufficient to meet the requirements of sulfide- or methane-oxidizing symbionts. However, species of vesicomyids have different physiological tolerances that determine their distributions within sites (Barry *et al.* 1997). A number of other seeps are known to fall within OMZs. In Monterey Bay, seeps at 800 and 1000 m support habitats similar to those described here, with only very rare siboglinid aggregations (Barry *et al.* 1997). At 800 m on the Pakistan margin, methane seepage within the OMZ core supports microbial mats and some small annelids, but no megafaunal specialists (Schmaljohann *et al.* 2001; C. Whitcraft, personal communication), although at sites below the OMZ there are seeps with *Calyptogena* sp. bivalves and siboglinid tubeworms (von Rad *et al.* 2000). Microbial mats associated with methane seepage at ~230 m in sulfidic regions of the Black Sea support dense assemblages of nematodes, foraminiferans and juvenile macrofauna, but no megafauna (Sergeeva & Gulin 2007). Vesicomyid clams as a group are an important exception. They seem highly adapted to living at low oxygen and high sulfide concentrations. Vesicomyids are one of the few chemosymbiont-bearing megafauna found at seeps in OMZs (Barry *et al.* 1997; Sahling *et al.* 2002).

OMZ effects on regional diversity

Where oxygen concentrations fall below 0.2 $\text{ml}\cdot\text{l}^{-1}$, OMZs appear to support distinct benthic species that are highly adapted to life at extremely low oxygen levels (Levin 2003; Gooday *et al.* 2009, 2010; Levin *et al.* 2009). But in the NE Pacific, the OMZ sediments off CA and OR had few species unique to this setting (note they were not compared to margin fauna at deeper depths). Only four taxa were found exclusively in the OMZ setting, three polychaetes and a sipunculid. The dominant OMZ (800 m) taxa were widespread species – including nephtyid and cossurid polychaetes off CA and paraonid, cirratulid and nephtyid polychaetes and a cumacean off OR (Appendix S1). Possibly the bottom-water oxygen concentrations off CA and OR are simply not low enough to select evolutionarily for highly adapted species. The oxygen minimum zone off CA and OR, while relatively stable with low values of 0.2–0.3 $\text{ml}\cdot\text{l}^{-1}$, does not exhibit the dysaerobic or near- anaerobic oxygen concentrations characteristic of the regions off Mexico, Peru, Oman and Pakistan, the source of most of our understanding of OMZ macrofauna. Transect sampling in these areas has revealed strong O_2 effects on macrofaunal density and diversity below 0.15 $\text{ml}\cdot\text{l}^{-1}$, with diversity increasing dramatically at 0.2–0.3 $\text{ml}\cdot\text{l}^{-1}$ (Levin *et al.* 1991, 2000, 2002, 2009). Vercoutere *et al.* (1987), Mullins *et al.* (1985) and Thompson *et al.* (1985) reported OMZ effects on benthos off CA, examining transect data for different size groups of animals than in the present study. They noted maximal faunal densities, polychaete burrows and tubes at the upper OMZ boundary, which they cited as 525 m off Pt. Sur, CA.

Global comparisons and threats

The macrofaunal densities recorded at 500 m on the CA and OR margin (10,000–14,000 $\text{ind}\cdot\text{m}^{-2}$) were higher than those reported from comparable depths off southern California (Hyland *et al.* 1991; Vetter & Dayton 1998) and Peru (Levin *et al.* 2002), but comparable to those reported in the OMZ on the Oman margin at 400 m (12,363 $\text{ind}\cdot\text{m}^{-2}$)(Levin *et al.* 2000). Similarly, CA 800 m (OMZ) densities (10,000 $\text{ind}\cdot\text{m}^{-2}$) were comparable to some other margins with OMZs (Peru – Gutiérrez *et al.* 2008; Chile – Gallardo *et al.* 2004) and without OMZs (*e.g.*, off North Carolina and South Carolina; Blake & Grassle 1994; Blake & Hilbig 1994). In contrast, densities reported in the OMZ off OR at 800 m (4269 $\text{ind}\cdot\text{m}^{-2}$) were lower than on other margins except the Pakistan margin, where oxygen levels were lower (Levin *et al.* 2009). The macrofaunal densities we recorded at Hydrate Ridge (OR) seeps were higher than those recorded by Sahling *et al.* (2002) for the same location, but he used a larger (0.5-mm) mesh size. Hydrate Ridge seep densities were comparable to macrofaunal densities in the deep Florida Escarpment microbial mats (3260 m) but much higher than those on the Aleutian margin (4450 m and 3280 m) (Levin & Mendoza 2007).

The margin settings we have studied are already subject to increased fishing pressure and to changing climate regimes. Sablefish (black cod), rockfish, sole and other flatfish are abundant in both regions studied, and are actively fished on the upper slopes of Northern California. Trawling acts to reduce structural heterogeneity in shelf and slope ecosystems around the world (Thrush & Dayton 2002). Gas hydrate mining is not yet economically feasible but pilot extraction studies are underway in Canada. Should hydrates be safely and economically mined, the Eastern Pacific and other margins will be faced with a host of new anthropogenic impacts. Understanding roles of habitat heterogeneity in generating and maintaining diversity will aid management and conservation of these upper slope environments. Heterogeneity has a unique role to play in the development of regulations for living resource and energy extraction and for the designation of deep-water protected areas.

Summary

The majority of biological studies of chemosynthetic ecosystems focus on within-system patterns and rarely examine the relationship of the fauna to the 'ambient' deep-sea environment. In the NE Pacific at upper slope depths (500–800 m) we find that over 40% of the heterotrophic seep macrofauna are shared with the non-seep and near-seep slope environments. Seeps support higher diversity than the non-seep margin and OMZ settings, and are very spatially heterogeneous themselves. Within a given depth zone (500 or 800 m), clam beds exhibited the highest diversity, possibly due to increased substrate heterogeneity and to geochemical heterogeneity induced by animal activities such as pumping of sulfate (Wallman *et al.* 1997; Treude *et al.* 2003). Increased stress reduces heterogeneity and the likelihood of specialized faunas. The high-stress settings with low bottom-water oxygen (OMZ) or high sulfide concentrations (microbial mat) exhibit high dominance and support a subset of those taxa found in the other settings examined. In the present study it was not possible to distinguish the potentially strong contribution of geographical setting to observed diversity patterns at seeps because it was confounded by differences in water depth, fluid flow regime and overlying oxygen conditions. Finally, the OMZ does not appear to dampen seep influence on diversity, although it is possible that the highly sulfidic clam bed habitats at Hydrate Ridge would support a greater diversity of species if they were not overlaid by an OMZ. Given the widespread distribution of gas hydrates at bathyal depths along continental margins, many undiscovered seeps undoubtedly exist within OMZs. Exploration of these settings, and of seeps below OMZs will shed light on the ways that oxygen, sulfide and methane interact in the environment to enhance the diversity we see on continental margins.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Macrobenthic densities (no. individuals per square meter, standard error) in upper margin, oxygen minimum zone (OMZ), clam bed, microbial mat and near-seep habitats on the California and Oregon margin.

Appendix S2 Percent of species shared between habitats (within geographic regions).