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Recruitment patterns of invertebrates at anhydrite hydrothermal vents on the Juan de Fuca Ridge, NE Pacific

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Abstract: Increased understanding of recruitment dynamics of vent species can provide greater insights into the variability in adult populations and aid in identifying factors, such as physical and/or environmental stressors, which may obscure the temporal history of recruitment patterns. At anhydrite vents within the ASHES vent field (Axial Volcano, Juan de Fuca Ridge) the infrastructure is unstable, fluids contain low metal but high sulphide concentrations, and temperatures range from 10 to 200°C. Despite high levels of larval supply to these vents, there is a paucity of colonizing invertebrates, either because of the physical instability of the anhydrite substratum or the chemical composition of the fluid. Colonization panels were used to quantify recruitment to three anhydrite vents (Virgin, Virgin's Daughter, Marshmallow) in the ASHES vent field over different time scales (1 vs. 2 yrs). Recruitment to anhydrite habitats did occur, but at lower levels compared to other vents in the field. The most abundant taxa colonizing the panels were gastropods and polychaetes. The recruit assemblages on the colonization panels generally were similar to the established assemblages inhabiting these vents, particularly after 2-yr periods. These results suggest that environmental factors, such as the temperature and/or chemistry of the vent fluid, rather than the physical instability of the anhydrite substratum are regulating invertebrate populations at anhydrite hydrothermal vents.

Keywords: Anhydrite ● Recruitment ● Axial volcano ● Gastropods ● Polychates ● Temperature ● Hydrothermal vents

Introduction

Populations of benthic invertebrates in most marine habitats are established and maintained through the influence of a suite of physical and biological mechanisms (Raimondi, 1990). However, such processes are not well understood for species that inhabit the patchy and ephemeral hydrothermal vent habitat. Survival and maintenance of these fragmented populations is partly dependent on larval dispersal, as well as successful recruitment. It is well established that variation in recruitment can affect the number and condition of adults available at time of reproduction (Connell, 1985). Thus, the quantification of recruitment dynamics in vent species can provide greater understanding of the variability in adult populations. It can also allow the identification of other factors, such as competition, predation, and environmental stressors, which may obscure the temporal history of recruitment patterns.

To date, there have been few studies at hydrothermal vents that have measured recruitment experimentally, using settlement panels (Van Dover et al., 1988; Mullineaux et al., 1998, 2000 & 2003; Hunt et al., 2004). All these studies have measured recruitment in basalt and sulphide chimney habitats, but to our knowledge, no studies have investigated invertebrate recruitment to anhydrite habitats.

The ASHES vent field at Axial Volcano, Juan de Fuca Ridge, contains several high-temperature (> 200°C) vents, multiple areas of diffuse venting, and several hot, vapour-phase chimneys composed entirely of anhydrite ($CaSO_4$) (Pruis & Johnson, 2004). These fragile chimneys have little infrastructure, and vent fluids with low metal but high sulphide concentrations (Tunnicliffe & Juniper, 1990). Unlike the large, solid sulphide chimneys within the same vent field, there are often few to no dense invertebrate assemblages associated with the surface of these anhydrite chimneys. There are, however, individual organisms observed around the base of some of these chimneys or within the mound of debris (pers. obs.). Tunnicliffe & Juniper (1990) suggested that vent organisms are not able to maintain their position closer to the venting fluid because of the instability of the substratum. Anhydrite solubility is temperature dependent, and large spires continuously form and collapse through precipitation and dissolution, varying the availability of a primary substrate for colonization at these habitats on a scale of days. However, it is also possible that the paucity of invertebrates at these sites is regulated by the chemical composition of the fluid.

In this study, we quantified recruitment to three anhydrite vents in the ASHES vent field over a two-year period by deploying colonization panels. We compared the recruit community to the established assemblages inhabiting these vents. We hypothesized that the availability of substrate is not the main factor that prevents the establishment of invertebrate communities at anhydrite vents, if the recruit and established assemblages are similar.

Materials and Methods

Recruitment was measured at 3 anhydrite vents separated by 10-12 m (Virgin, Virgin's Daughter and Marshmallow) in the ASHES vent field (45°55'N, 130°03'W; depth = 1546 m) at Axial Volcano on the Juan de Fuca Ridge, between 2001 and 2003 by deploying colonization panels using the ROV ROPOS. Recruitment was defined as the accumulated number of metamorphosed individuals on the panels at the end of the recovery period. Panels of solid basalt (~ 7 cm x 10 cm x 2 cm) were used as substrate to mimic natural vent substrates. They were held in plastic containers with holes on the underside, allowing for the diffusion of fluid through the plastic container around the panel. For ease of deployment and recovery, 5 replicate panels for each vent were attached to a galvanized steel frame, using 20-cm galvanized steel threaded rods covered by smooth polyethylene tubes. Frames were deployed: (1) at Virgin in 2001, retrieved in 2002 (353 d), (2) at Virgin's Daughter in 2001, retrieved in 2003 (764 d) and (3) at Marshmallow in 2002, also retrieved in 2003 (411 d). At each vent, the frame was positioned directly over the opening, so that all settlement panels were in similar contact with the vent fluid (Fig. 1). At Virgin, the plastic containers, steel rods, and parts of the steel frame disintegrated from melting, resulting in the loss of 1 of the 5 settlement panels.

For recovery, each frame was carefully placed in a LEXAN lidded box (~ 80 cm x 60 cm x 35 cm), previously positioned within 0.5-1 m of the frame on the ocean floor. On the underside of the box's lid, a glued 5-cm layer of open cell foam ensured a snug fit preventing the dislodgment of organisms from the panels during transport. Upon recovery, individual panels (and the corresponding section of foam overlying the panel) were removed from the frame, frozen at -80°C aboard ship and then transferred into 95% ethyl alcohol for long-term storage. In 2003, we collected one suction sample of the established assemblage from the vent base at Virgin's Daughter and Marshmallow. Samples were fixed in 10% seawater formalin for 48 hours before transfer to 95% ethyl alcohol for long-term storage. All invertebrates were counted under a light microscope and identified to the lowest possible taxonomic level based on morphology.

Differences in recruitment of the invertebrate assemblage to the colonization panels between 2001-2002 and 2002-2003 were explored with 1-way (random factor: time) MANOVA. A 1-way ANOVA (fixed factor: vent) was also used to test for differences between all 3 vents in abundances of species found on more than one vent, followed by Tukey's HSD post-hoc test. For Virgin's Daughter and Marshmallow, the similarity in the composition of the background and colonization assemblages was examined using the Bray-Curtis Similarity Coefficient, with Primer v5 (PRIMER_E Ltd.). For each vent, the abundance of all species was pooled across the 5 replicate colonization panels to obtain a total colonization assemblage. Because the sampling area of established assemblages could not be quantified, we used relative abundance of each species (abundance of species/total abundance of all species in the sample) for this analysis. Relative abundances were fourth-root transformed to similarly influence all levels of abundance (rare to high).

Temperature was measured at Virgin at 30 min-intervals between July 2001 and July 2002 with a HOBO temperature probe. For Virgin's Daughter and Marshmallow, point measures were taken with the ROPOS temperature probe during panel recovery. Vent flow vigour and the presence of vent fauna were observed visually upon panel deployment and recovery.

Figure 1. *In situ* photograph of the colonization panels being deployed around the anhydrite chimney at Virgin vent, ASHES vent field, in 2001 by the ROV ROPOS.

Figure 1. Photographie *in situ* des plaques de colonisation autour des cheminées anhydres à Virgin, site de ASHES, en 2001, prises par le ROV ROPOS.

Results

The physical environment at Virgin was more dynamic than at Virgin's Daughter and Marshmallow. At Virgin, a large anhydrite spire (50-60 cm) and mound (75-80 cm in radius) were present. White fluid was venting extremely vigorously from the centre of the orifice. In contrast, at Marshmallow and Virgin's Daughter, anhydrite spires and mounds were absent and a thin anhydrite layer covered the basalt near the orifice. The observed shimmering flow was more intense at Virgin's Daughter than Marshmallow. The hottest temperatures were recorded at Virgin, and ranged from 270 to 290°C (Fig. 2). There was little fluctuation throughout most of the year of deployment, although there was a slight decrease of 15°C from July 2001 to July 2002. Temperatures measured directly over the panels (~ 25 cm above vent opening) at Marshmallow and Virgin's Daughter were 8-16ºC and 11-17ºC, respectively. At the vent opening, temperatures were 29-35ºC and 19-24ºC, at each of the two vents respectively.

A total of 13 taxa colonized the panels, and 4 were present at all vents (Table 1). Of these, the gastropod *Lepetodrilus fucensis* McLean 1988 was the most abundant (Fig. 3). Abundances of the gastropods *Depressigyra globulus* Warén & Bouchet, 1989 and *Provanna variabilis* Warén & Bouchet, 1986, and of the polychaete *Amphisamytha galapagensis* Zottoli, 1983 were of the same order of magnitude (Fig. 3). Six taxa occurred at only one vent (Table 1). There were no significant temporal differences in the abundance of recruits between 2001-2002 and 2002-2003 (MANOVA: Wilk's $\lambda = 0.581$, $F_{4,4} = 0.720$, $p = 0.621$). Significantly higher abundances of *L. fucensis*

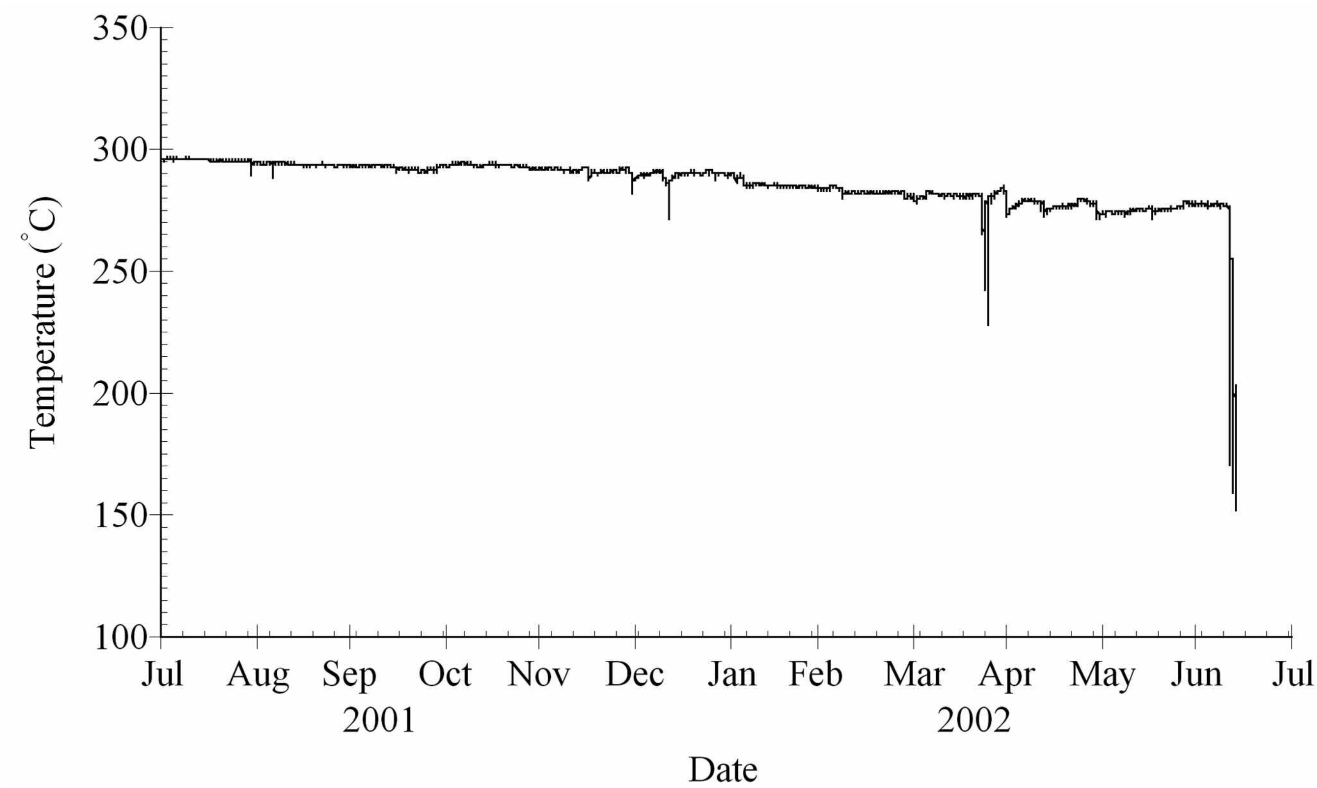

Figure 2. Temperature (°C) recorded at Virgin between 2001 and 2002, as measured with a HOBO temperature probe inserted directly into the venting fluid. Data provided by R.W. Embley (NOAA VENTS program).

Figure 2. Température (°C) enregistrée à Virgin entre 2001 et 2002 par une sonde HOBO directement soumise au fluide de la source. Données fournies par R.W. Embley (programme NOAA VENTS).

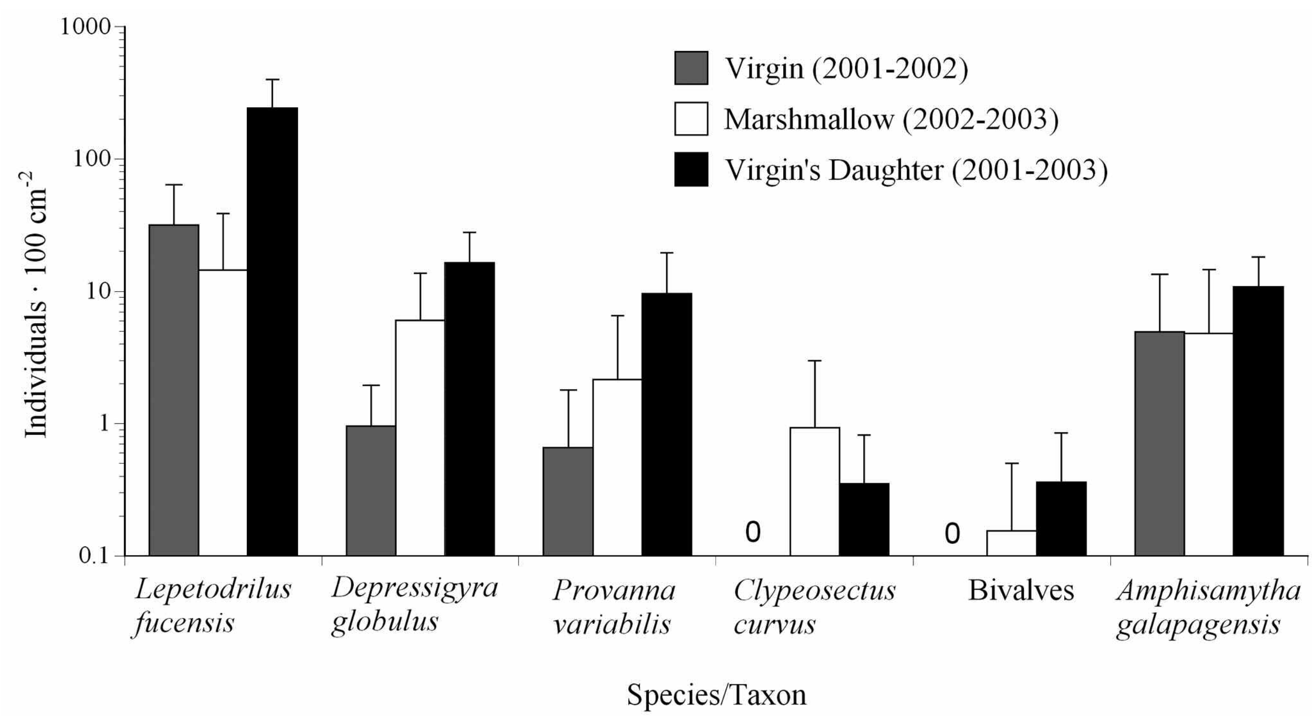

Table 1. Relative abundance (RA) of taxa in established and colonization assemblages at anhydrite vents at Axial Volcano. Bray-Curtis similarity coefficients were calculated between the two types of assemblages collected from Virgin's Daughter and Marshmallow vents. V = Virgin vent; VD = Virgin's Daughter vent; M = Marshmallow vent. Numbers in parentheses indicate number of replicate colonization panels pooled for RA calculation.

Tableau 1. Abondance relative (RA) des taxons des assemblages en place et sur les plaques de colonisation au niveau des sources anhydres à Axial Volcano. Les coefficients de similarité de Bray-Curtis sont calculés entre les deux types d'assemblages récoltés sur les sites Virgin's Daughter et Marshmallow. V = Virgin ; VD = Virgin's Daughter ; M = Marshmallow. Les nombres entre parentheses indiquent le nombre de plaques de colonisation (réplicats) rassemblés pour le calcul de RA.

Taxon	*Species*	***% RA Established***		***% RA Colonization Panels***		
		VD	**M**	**V (4)**	**VD (5)**	**M (5)**
Gastropoda	*Lepetodrilus fucensis*	81.7	21.1	81.1	83.8	52.3
	Depressigyra globulus	7.5	59.2	4.1	5.9	19.1
	Provanna variabilis	5.8	0.4	1.6	3.4	7.0
	Clypeosectus curvus	0	0	0	0.1	3.0
	Pyropelta musaica	0.4	0	0	0.3	0
Polychaeta	*Amphisamytha galapagensis*	3.3	0.4	12.3	3.9	15.6
	Paralvinella sulfincola	0	13.5	0	0	0
	Nereis piscesae	0	0	0	0.1	0
	Lepidonotopodium piscesae	0.8	0.9	0	0	0
	Levensteiniella kincaidi	0	0.4	0	0	0
	Branchionotogluma sp.	0	0.9	0	0	0
	Unidentified polychaetes*	0	0	0.8	0.1	1.0
Bivalvia	Bivalve sp.	0	0	0	0.1	0.5
Ostracoda	Ostracod sp.	0	3.1	0	1.9	0
Copepoda	Copepod sp.	0	0	0	0	0.5
Nematoda	Nematode sp.	0.4	0	0	0	1.0
Acarina	*Copidognathus papillatus*	0	0	0	0.4	0
Bray-Curtis similarity coefficient		73.1	47.0			

*most likely *A. galapagensis*

($F_{2, 11} = 12.27$, $p = 0.002$) and *D. globulus* ($F_{2, 11} = 6.67$, $p = 0.013$) recruits were found on colonization panels at Virgin's Daughter after 2 years than at Virgin and Marshmallow after only 1 year. There were no significant differences found in the level of recruitment among vent/year combinations for any of the other species on the colonization panels. It is possible that a few individuals of mobile species may have migrated onto the panels during deployment. However, most colonists recovered from the panels were small, juvenile stages, implying their origin as settlement to the panels rather than emigration.

The established assemblages at these anhydrite vents included 11 taxa. The most abundant species were *L. fucensis* at Virgin's Daughter and *D. globulus* at Marshmallow (Table 1). Overall, gastropods and polychaetes were the most abundant taxa. The colonization and established assemblages shared 5 taxa at Virgin's Daughter and 4 at Marshmallow. The unidentified polychaete listed in Table 1 preserved in poor condition, preventing accurate identification from morphology, but was most likely *A. galapagensis*. The Bray-Curtis similarity coefficient between colonization and established assemblages was 73.1 and 47.0 for Virgin's Daughter and Marshmallow, respectively.

Discussion

Recruitment of vent invertebrates to colonization panels occurred at all three anhydrite vents, but was lower than at other vents within the ASHES vent field (Kelly, unpublished data). Low recruitment levels lead to low abundance of adult populations and can be the result of either low larval availability or high mortality during

Figure 3. Abundance of recruits of invertebrates collected on basalt colonization panels at Virgin, Virgin's Daughter and Marshmallow vents between 2001 and 2003. Error bars are standard deviations ($n = 5$, except at Virgin, where $n = 4$).

Figure 3. Abondance des recrues d'invertébrés récoltées sur les plaques de colonisation en basalte à Virgin, Virgin'Daughter et Marshmallow entre 2001 et 2003. Les barres d'erreur correspondent aux écart-types ($n = 5$, sauf à Virgin où $n = 4$).

settlement. At Axial Volcano, larval abundance in the water column above the vent fields did not vary spatially over scales of m to km, suggesting the presence of a homogeneous larval pool within the floor of the caldera (Metaxas, 2004). Using passive larval collectors, Metaxas (2004) also showed that larval availability near the benthos was similar at Virgin as at other vents within ASHES. As in this study, gastropods (especially *L. fucensis*) and polychaetes were the most abundant larval taxa, reflecting the relative abundances of recruits on the colonization panels. Thus, we suggest that low recruit abundance is not related to low larval supply. More likely, mortality at or after settlement is influencing the observed recruitment patterns. Post-settlement biological processes, such as bulldozing by grazers and competition for space are possible but unlikely, given the low species abundances observed on the panels. Thus, we propose that environmental stressors, arising from the properties of the venting fluid (temperature, pH, flow variability and metal and sulphide concentrations), are the most likely source of mortality for settling larvae at anhydrite vents.

The observed similarity between the established and colonizing assemblages also suggests that fluid chemistry has the greatest influence on abundance. Colonization panel assemblages were more similar to the established assemblages at Virgin's Daughter, than at Marshmallow. We hypothesized that a great similarity between the two assemblages would indicate that the availability of suitable substrate is not regulating species assemblages at these sites. Our results suggest that the role of availability of primary substrate may vary among anhydrite vents with similar chemical characteristics.

Differences between established and colonization panel assemblages can arise from the different collection methods used for each assemblage. Many small faunal species within the established assemblage could not be collected as efficiently as from colonization panels because the mesh size on the suction sampler precluded the collection of individuals < 250 µm. In contrast, the colonization panels had many small holes, cracks and crevices that small individuals could occupy. However, there is no reason to assume that this difference is vent-specific.

The observed patterns in similarity between colonizing and established assemblages likely are affected most strongly by proximity to the vent fluid. Colonization panels at Virgin's Daughter and Marshmallow were exposed to similar temperatures, although lower than those recorded directly at the openings at both vents. The difference in temperature between the vent opening and the panels was greater at Marshmallow than Virgin's Daughter. At Marshmallow, the hotter temperatures directly at the vent may restrict or altogether inhibit the recruitment of species with lower temperature tolerances. For example, *Paralvinella sulfincola* Desbruyères and Laubier 1986, is known to prefer high temperature environments (Sarrazin et al., 1997), and was only collected in the established assemblage. *L. fucensis* and *D. globulus* ranked as the first and second most abundant species on colonization panels, but this trend was reversed in the established assemblage. *L. fucensis* has a lower thermal tolerance (30-35ºC) than *D. globulus* (35-40ºC) (Lee, 2003).

Alternatively, the longer deployment at Virgin's Daughter than at Marshmallow may be responsible for the observed similarity patterns between established and colonization panel assemblages. Previous studies have recorded significant recruitment to artificial panels (Van Dover et al., 1988; Mullineaux et al., 1998) or new vents (Tunnicliffe et al., 1997) within one year, but a 'mature' vent assemblage may take several years to develop (Shank et al., 1998). Species assemblages on the panels at Virgin's Daughter may reflect a more advanced stage in community evolution and would thus be more similar to the established assemblages measured near the vent opening. Lending further support to this suggestion, abundances of *L. fucensis* and *D. globulus* were greater on colonization panels at Virgin's Daughter than at the other anhydrite vents.

Virgin provides a much hotter, more hostile environment than either Marshmallow or Virgin's Daughter, as it has a chimney composed of porous, fragile anhydrite, which collapses and re-forms through dissolution and precipitation on time scales of days to weeks (Tunnicliffe & Juniper, 1990). While we did not collect samples of the adult assemblage at Virgin vent upon recovery, photographs of the frame *in situ* were taken in 2000 and 2001. These indicated that the established community was sparsely (in terms of abundance and biomass) populated by 1-2 species (*Paralvinella* sp.). Similarly, only four species were recovered from colonization panels at Virgin (*L. fucensis*, *D. globulus*, *P. variabilis*, and *A. galapagensis*), which occurred altogether on only 1 of the 4 panels, while *L. fucensis* and *D. globulus* were the only species found on the other 3 panels. Also, the total number of individuals collected from colonization panels at Virgin was ~ 8% and ~ 60% of that at Virgin's Daughter and Marshmallow, respectively. The temperature of the fluid is likely too high to allow settlement of invertebrates, as the upper limit of metazoan life is currently considered to be 45-55ºC (Lee, 2003). Temperatures at Virgin seldom fell below 200ºC between 2001-2002, and their observed melting indicated that the temperature was also extremely hot at the height of colonization panels.

This is the first study to measure recruitment of invertebrates at anhydrite vents, as well as explore some of the factors influencing species abundances at these vents. By providing basalt substrates, we showed that colonizing assemblages of benthic invertebrates at anhydrite vents generally are similar to the established assemblages. These results and previous studies suggest that neither larval supply nor a lack of substrate availability is regulating invertebrate populations at anhydrite vents. It is most likely that chemical conditions at these vents have a pronounced effect on the assemblages.

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