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Tubeworm succession at hydrothermal vents: use of biogenic cues to reduce habitat selection error?

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Abstract Species colonizing new deep-sea hydrothermal vents along the East Pacific Rise show a distinct successional sequence: pioneer assemblages dominated by the vestimentiferan tubeworm *Tevnia jerichonana* being subsequently invaded by another vestimentiferan *Riftia pachyptila*, and eventually the mussel *Bathymodiolus thermophilus*. Using a manipulative approach modified from shallow-water ecological studies, we test three alternative hypotheses to explain the initial colonization by *T. jerichonana* and its subsequent replacement by *R. pachyptila*. We show that *R. pachyptila* and another vestimentiferan, *Oasisia alvinae*, colonized new surfaces only if the surfaces also were colonized by *T. jerichonana*. This pattern does not appear to be due to restricted habitat tolerances or inferior dispersal capabilities of *R. pachyptila* and *O. alvinae*, and we argue the alternative explanation that *T. jerichonana* facilitates the settlement of the other two species and is eventually outcompeted by *R. pachyptila*. Unlike the classic model of community succession, in which facilitating species promote their own demise by modifying the environment to make it more hospitable for competitors, we suggest that *T. jerichonana* may produce a chemical substance that induces settlement of these competitors. This process of selecting habitat based on biogenic cues may be especially adaptive and widespread among later-successional species that occupy a physically variable and unpredictable environment. In these cases, the presence of weedy species implies some integrated period of environmental suitability, whereas an instantaneous assessment of physical habitat conditions, such as water temperature for vent tubeworms, provides a poorer predictor of long-term habitat suitability.

Key words Deep-sea ecology · Hydrothermal vent · *Riftia pachyptila* · Succession · *Tevnia jerichonana*

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Introduction

The unexpected encounter with hydrothermal vent communities on the deep Pacific seafloor in 1977 (Corliss et al. 1979) remains one of the most fascinating biological discoveries of the century. Over the past two decades, we have learned that these productive communities of unique species are fueled largely by chemosynthetic microbial production, and that they sustain their populations despite the patchy, ephemeral nature of the vent habitat. We know little, however, about what processes organize vent communities and control temporal and spatial patterns of species composition.

Vent faunas display striking spatial patterns, with distinct species assemblages replacing each other along a spatial gradient of hydrothermal fluid flux. At one extreme of this gradient, in focused "black smoker" vents, hydrothermal fluids reach temperatures of 403°C, and contain concentrations of H_2S (an important reduced substrate that provides the chemical energy source for chemosynthetic microbial production) of up to 30 mmol kg^{-1} (Johnson et al. 1988). Diluted vent fluids are also released from the seafloor after mixing with ambient seawater. Because the high-temperature fluids are characterized by low pH and potentially toxic concentrations of both H_2S and heavy metals, a gradient in fluid flux presents not only a gradient in energy resources, but also a gradient in physico-chemical stresses to the organisms exposed to them.

At hydrothermal vents on the East Pacific Rise, a distinct biological zonation occurs along the fluid flux gradient, with alvinellid polychaetes inhabiting areas of high-temperature flows (>25°C above ambient tempera-

ture), vestimentiferan tubeworms occurring in vigorous diffuse flows (up to 25°C), bivalves in moderate diffuse flows (up to 10°C) and various suspension feeders, including barnacles and serpulid polychaetes, in weak diffuse flows (<2°C) (Hessler et al. 1985). This correspondence between physico-chemical gradients and faunal zones has led vent biologists to focus on hydrothermal fluid flux as the main factor organizing vent communities (Childress and Fisher 1992). Indeed we know that the prominent vestimentiferan *Riftia pachyptila* and the vent bivalve *Calyptogena magnifica* have very specific requirements for their physical and chemical environment, based on their physiological tolerances and the need to provide H_2S to their endosymbionts (reviewed in Childress and Fisher 1992).

Physiological tolerances and nutritional requirements, however, are unlikely to be the sole determinants of community structure at vents. Population densities, biomass, and spatial cover of benthic invertebrates are typically very high in vent communities, implying a potential for interactions among species. Correlations between vertical gradients in physical stress and population distributions in the rocky intertidal habitat had long been interpreted as evidence that vertical biological zonation was largely a consequence of differing adaptations to physiological stressors (e.g., Lewis 1964). Subsequent experimental manipulations, however, including relatively simple experiments feasible even in the deep sea, demonstrated the significance of biological factors such as competition, predation, and biological disturbance in setting limits to species distributions (e.g., Connell 1961, 1972; Paine 1966; Dayton 1971; Sousa 1979; Paine and Levin 1981). The lesson learned from these studies is that a significant correlation with physico-chemical factors provides insufficient evidence for inferring causation of zonation along an environmental gradient. Experimental manipulations provide the most unambiguous means of separating the contributions of physiological tolerances and biological interactions (Paine 1977).

Analogous to the prevailing explanations for spatial zonation, the successional patterns at hydrothermal vents have also been interpreted in terms of species' responses to changes in the character of hydrothermal vent flux. At newly opened vents along the East Pacific Rise, the initial visibly dominant sessile metazoan in vigorous diffuse flow regions appears to be the small vestimentiferan *Tevnia jerichonana* (Lutz et al. 1994; Shank et al. 1998). This species is then replaced by the larger species *Riftia pachyptila*, frequently over a period of less than 1 year. Later in the sequence, the mussel *Bathymodiolus thermophilus* colonizes and may, in some cases, displace the vestimentiferans (Hessler et al. 1988). A third, small, vestimentiferan species, *Oasisia alvinae*, is also found sporadically in this environment. It is not known when in the temporal sequence this species colonizes vents because it is not easily distinguished from *T. jerichonana*.

In this study, we examine the relative roles of physical and biological processes early in the succession from a *T. jerichonana*-dominated towards a *R. pachyptila*-dominated assemblage in the vigorous diffuse-flow zone of hydrothermal vents on the East Pacific Rise. We explore three alternative hypotheses to explain this succession (as defined broadly by Connell and Slatyer 1977) at vents. The first possibility is that species composition changes over time as individual species respond independently, because of their differential tolerances and nutritional requirements, to progressive changes in fluid flux or composition as the vent ages. The second is that successions of species are due to intrinsic species differences in dispersal capabilities of their larvae. The third is that species changes are due to facilitation of the secondary species by the pioneer species, which is then outcompeted. The first hypothesis was proposed by Shank et al. (1998), and is a logical extension of the idea that the distribution of each vent species is fully controlled in time and space by its response to variations in the physical and chemical environment. The second hypothesis supposes that larvae of *T. jerichonana* are more consistently available and/or are better at dispersing and settling into vent habitats, thereby making this species a weed among vestimentiferans. In terrestrial plant communities, differing dispersal characteristics have been shown to determine which species are able to first colonize newly opened habitats (Eriksson 1996). The third hypothesis supposes that *T. jerichonana* precedes *R. pachyptila* because the latter species does not effectively colonize without facilitation by the former, and that subsequently *T. jerichonana* is outcompeted by *R. pachyptila*. This scenario of an initial facilitative species being eventually outcompeted by the species it facilitates is a well-known phenomenon, and is the essential ingredient in the classic Odum (1969) model of succession in plant communities (reviewed in Callaway 1995).

Predictions derived from these hypotheses were tested using field colonization assays. In the first scenario, we would expect that initial (pioneer) species would not colonize habitats that were in later stages of succession (because the physical/chemical environment would have changed to become unsuitable for the pioneer). In the second scenario, we would expect colonization of each species to be primarily dependent on the timing (or spatial distribution) of larval availability in the water column. In the third scenario, we would expect colonization of *R. pachyptila* always to follow colonization by *T. jerichonana*, regardless of the physico-chemical status of the habitat, or the timing of larval availability.

Methods

We tested our competing hypotheses by quantifying colonization sequences of vestimentiferans on cubic basalt blocks roughly 10 cm on a side. The studies were conducted using the submersible *Alvin* at vents near 9°50'N, 104°17'W along the East Pacific Rise (Fig. 1) at a water depth of 2500 m. This location was selected because nearby vent communities had been monitored frequently since 1991 when a well-documented volcanic event had occurred (Haymon et al. 1993). For each of three deployment periods, replicate (n=3) blocks were introduced into three separate vestimentiferan tubeworm clumps at each of three vent fields

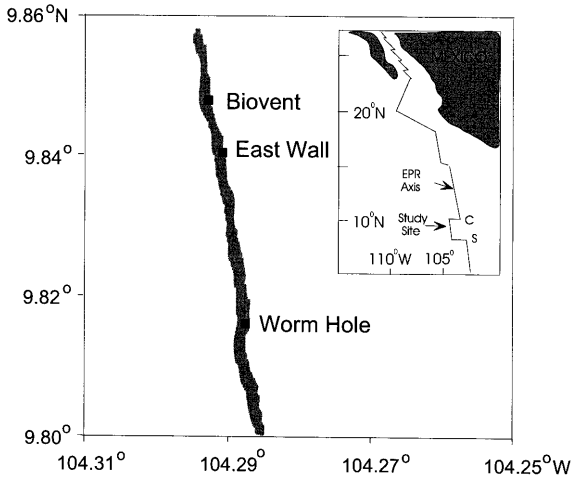

Fig. 1 Map of Biovent, East Wall, and Worm Hole vent sites, located on the axis of the East Pacific Rise (*shaded line*) near 9°50'N, East Pacific Rise, between the Clipperton (*C*) and Sequieros (*S*) fracture zones

(Worm Hole, East Wall, and Biovent) located along a 2-km section of the axial valley. These vent fields appeared to be at different stages in the colonization sequence: Worm Hole was dominated by abundant clumps of *T. jerichonana* with no co-occurring mussels and scattered individual *R. pachyptila*; East Wall was co-occupied by *T. jerichonana* and *R. pachyptila* (the clear dominant in numbers and biomass) but with no co-occurring mussels; and Biovent was inhabited by only one evident tube worm, *R. pachyptila*, along with co-occurring vent mussels.

As part of a predation-exclusion study not described here, we deployed additional basalt blocks suspended inside cages measuring 20 cm on a side and covered with 7-mm plastic mesh, and others suspended inside cage-controls (identical to the cages, but lacking the mesh on one side). In order to attain a sufficient sample size for the succession analyses, results from caged blocks are included in our results, but the potential effects of caging on the species composition of vestimentiferan colonists are considered before inclusion of these data in any analysis.

The fluid microhabitat of each block was characterized on deployment and recovery by taking temperature measurements with the *Alvin* temperature probe at the lowermost extremity of the block or cage. This position was chosen because it was typically closest to the fluid source and offered a consistent measure to compare among blocks. Occasional temperature underestimates were possible using this technique because if the probe was not fully extended, it could record in the more dilute, lower-temperature fluids. Although the probe measurements did not describe the complete temperature environment over all surfaces of the block, they did provide a quantitative measure for ranking the block environments along a temperature gradient.

Temperatures were recorded as the maximum anomaly from ambient (1.8°C) observed during a single probe emplacement. The probe was maintained in place until the temperature measurements reached a plateau, which typically took approximately 10 s or less. Time-series measurements in the vent fluid flows at this study site showed that extended measurements (several minutes) did not produce significantly different results, so 10 s was selected as the minimum interval to give a representative reading. These temperature measurements were essential because spatial variation in vent fluid flux occurred even within a zone of relatively homogeneous biology, and by documenting the temperature associated with each block we were able to evaluate possible influences of temperature on colonization. For information on longer-term (months) temperature variation, measurements were obtained with a single internally-recording "Hobo" temperature probe at each site for the duration of the block deployments.

Temperature anomaly was used as a proxy for both physiological stress from exposure to high temperature, H_2S or toxic metals, and also nutritional benefit from chemosynthetic microbial production. Although the relationship between temperature and chemical composition of vent fluids can vary significantly among spatially separated vents (Von Damm 1995), temperature and chemistry are strongly correlated within a site (Johnson et al. 1988). We were not able to measure the chemical environment at each block, but water samples from selected sites indicated that H_2S concentration did vary as expected with temperature in our study area (authors, unpublished work).

The microenvironment of each block was characterized as suitable or unsuitable for vestimentiferan colonization based on temperature measurements and an assessment of the immediately adjacent fauna. Areal coverage of dominant space occupiers and abundance of consumers/predators (crabs and fishes) in the immediate vicinity of each block were documented semi-quantitatively via video images. Of the uncolonized blocks, only those that remained in a suitable microenvironment (defined as where the recorded temperature anomaly exceeded 2°C and where healthy vestimentiferans surrounded the block) were included in analyses. Because of the potential for underestimating temperature, the highest temperature measured at any one block was used to determine the suitability of its microenvironment.

Sets of blocks were deployed and recovered on three cruises (in November 1994, April 1995, and December 1995) in a series of overlapping time intervals (November 1994–April 1995=5 months; April 1995–December 1995=8 months; November 1994–December 1995=13 months). The purpose of these overlapping deployments was to test for positive as well as negative biological interactions, in the context of succession theory (Odum 1969; Sutherland and Karlson 1977). The rationale followed that of Ambrose (1984): if the sum of the colonists on blocks in the two consecutive (5- and 8-month) intervals is similar to the number in the continuous 13-month interval, then initial colonists would appear to have no detectable effect on later arrivals. If the sum of colonists across the consecutive intervals is less than the numbers accumulated over the continuous interval then positive, facilitative interactions would be indicated. If, however, the sum of the colonists in the consecutive intervals is greater, inhibitory interactions, such as preemption or interference competition for nutritional resources, would be suggested.

Vestimentiferan recruits on all blocks were enumerated under a dissecting microscope and, if possible, identified to species. A molecular genetic technique was developed to identify those individuals that were too small to ascribe to species using traditional morphological techniques. For molecular identification, individuals were collected directly from blocks and frozen at –80°C or were scraped from ethanol-preserved blocks. Frozen samples were homogenized with a plastic tissue grinder (Kontes 749515–0000) in 25–50 µl of digestion buffer (0.1 M NaCl, 0.05 M Tris-HCl, 0.01 M EDTA, 0.5% sodium dodecyl sulfate, and 0.1 mg ml^{-1} proteinase K) in a 1.5-ml microfuge tube. The volume of digestion buffer used depended on the size of the animal to be homogenized. Ethanol-preserved specimens were dried in a Speed Vac to remove ethanol from the sample prior to homogenization; then the procedure for frozen samples was followed. The homogenized samples were digested overnight at 42°C. The digested samples were extracted once with phenol/chloroform and DNA was purified with a Qiaquick column (Qiagen 28104). The 28S rRNA gene was amplified from the purified DNA sample with polymerase chain reaction (PCR). Two oligonucleotides were designed to amplify a 406 base pair (bp) fragment from the *Riftia pachyptila* 28S rRNA gene (GenBank Accession Z21543) (Williams et al. 1993). The 5' oligonucleotide begins at position 63 and ends at position 86 (5' TAA ACG GAT GGG A(AC)C GCA AAG TCG 3') and the complementary 3' oligonucleotide begins at position 468 and ends at position 448 (5' GAT TCG CCA CAG ACC CTG AGC 3'). A standard 100-µl PCR reaction included: 1 µl vestimentiferan DNA, 1*x* Boeringer Mannheim PCR buffer with Mg++, 80 pmol each of the two PCR oligonucleotides, 0.2 mmol l^{-1} dNTPs, and 2.5 units *Taq* polymerase (Boeringer, Mannheim). The amplification cycle was: denature 1 min at 94°C, anneal 2 min at 55°C, and

primer extension 2.5 min at 72°C, for a total of 30 cycles. PCR products were purified with a Qiaquick column. The PCR-amplified DNA was digested with the restriction endonuclease *Taq*I and the resulting DNA fragments were separated on 2% agarose electrophoresis gels in 1*x*TBE (0.089 mol l^{-1} Tris-HCl, 0.089 mol l^{-1} Borate, and 2.5 mmol l^{-1} EDTA). Species identifications for the three East Pacific Rise vestimentiferan species were made based on the predicted *Taq*I digestion patterns of the 28S rRNA amplification products: *R. pachyptila*, 180 bp, 133 bp, and 93 bp; *T. jerichonana*, 298 bp, 93 bp, and 8 bp (not visible); and *O. alvinae*, 182 bp, 115 bp, and 103 bp. The GenBank Accession numbers for the three species are: *R. pachyptila* (Z21534), *T. jerichonana* (Z21529), and *O. alvinae* (AF198625).

Results

Even for the shortest of the deployment intervals, we obtained remarkably high recruitment of vestimentiferans on the blocks (Table 1). Most of the vestimentiferan colonists, however, were less than 1 cm long, limiting our ability to identify them to species based on their morphology. Early recruits consisted of a basal capsule and, if big enough, a tube, but they had none of the morphological characters (Jones and Gardiner 1989) traditionally used to distinguish the species. Molecular identifications were performed on individual vestimentiferans from most of the recovered blocks, including representatives from all three sites and all three deployment intervals. Due to the very large number of small recruits, only subsamples of recruits from each block were identified with molecular methods. We attempted to identify at least 30 small individuals on each block, but DNA amplification of the ethanol-preserved specimens was variably successful, resulting in smaller sample sizes. On some blocks, the largest individuals of *R. pachyptila* could be distinguished by morphology from the other two species. However, because the other two species were not distinguishable by appearance and morphological identification was size-dependent, only those vestimentiferans identified by molecular tools were used in analyses of patterns requiring species-level data. There was no detectable difference in vestimentiferan species composition of colonists on caged versus uncaged blocks (Table 1), so all blocks from habitats characterized as suitable for colonization (based on maximum temperature and surrounding fauna, or presence of live vestimentiferan colonists) were included in our analyses.

Differences in vestimentiferan species composition among blocks did not appear to result from species-specific differences in response of colonists to small-scale variations in the physical (and inferred chemical) habitat within a vestimentiferan clump. We tested this possibility by comparing the species composition of colonists to the temperature anomaly associated with each block. All blocks from habitats characterized as suitable for colonization were included in this analysis. None of the vestimentiferan species showed a significant relationship between their relative abundance and the temperature environment of a block (Fig. 2). Temperatures on deployment and recovery were not substantially different for most blocks (Table 1), and a similar analysis using the averaged anomaly from the two temperature measurements also showed no temperature-related trend for any species. Time series measurements at each site showed little trend in the mean temperature over the block deployment intervals (although tidal variation on daily and fortnightly time scales was evident), and little difference among sites (Fig. 3). Thus, the physical/chemical environment of the blocks appeared to have been variable but not changing greatly in mean conditions over the duration of a deployment interval.

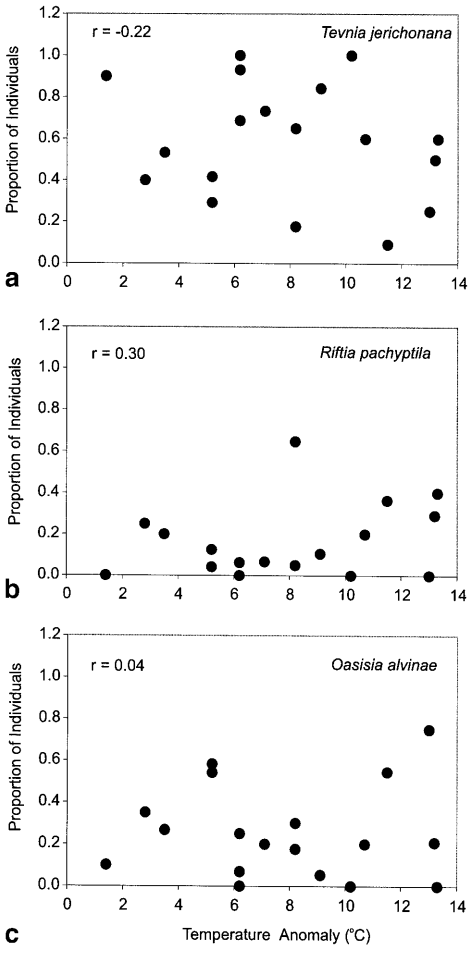

Fig. 2 Relation between relative abundances of each of the three vestimentiferan species, **a** *Tevnia jerichonana*, **b** *Riftia pachyptila*, and **c** *Oasisia alvinae*, and temperature microhabitat of each colonized basalt block. Only blocks with at least one genetically identified vestimentiferan colonist are shown (n=18 blocks for each species; 2 points in **b** are identical). Temperature is the maximum anomaly recorded from the base of each block or cage. Correlations between temperature and relative abundance were not significant (P<0.05) for any of the three species: Pearson's r=–0.22 for *T. jerichonana*, 0.30 for *R. pachyptila*, and 0.04 for *O. alvinae*. Note that correlations between temperature and absolute abundance also were non-significant (P<0.05) for all three species (Pearson's r=–0.12 for *T. jerichonana*, 0.30 for *R. pachyptila*, and –0.12 for *O. alvinae*), so the lack of correlation in relative abundances is not likely due to use of proportions

Neither spatial nor temporal restriction of larvae appeared to imprint pattern upon the colonization data

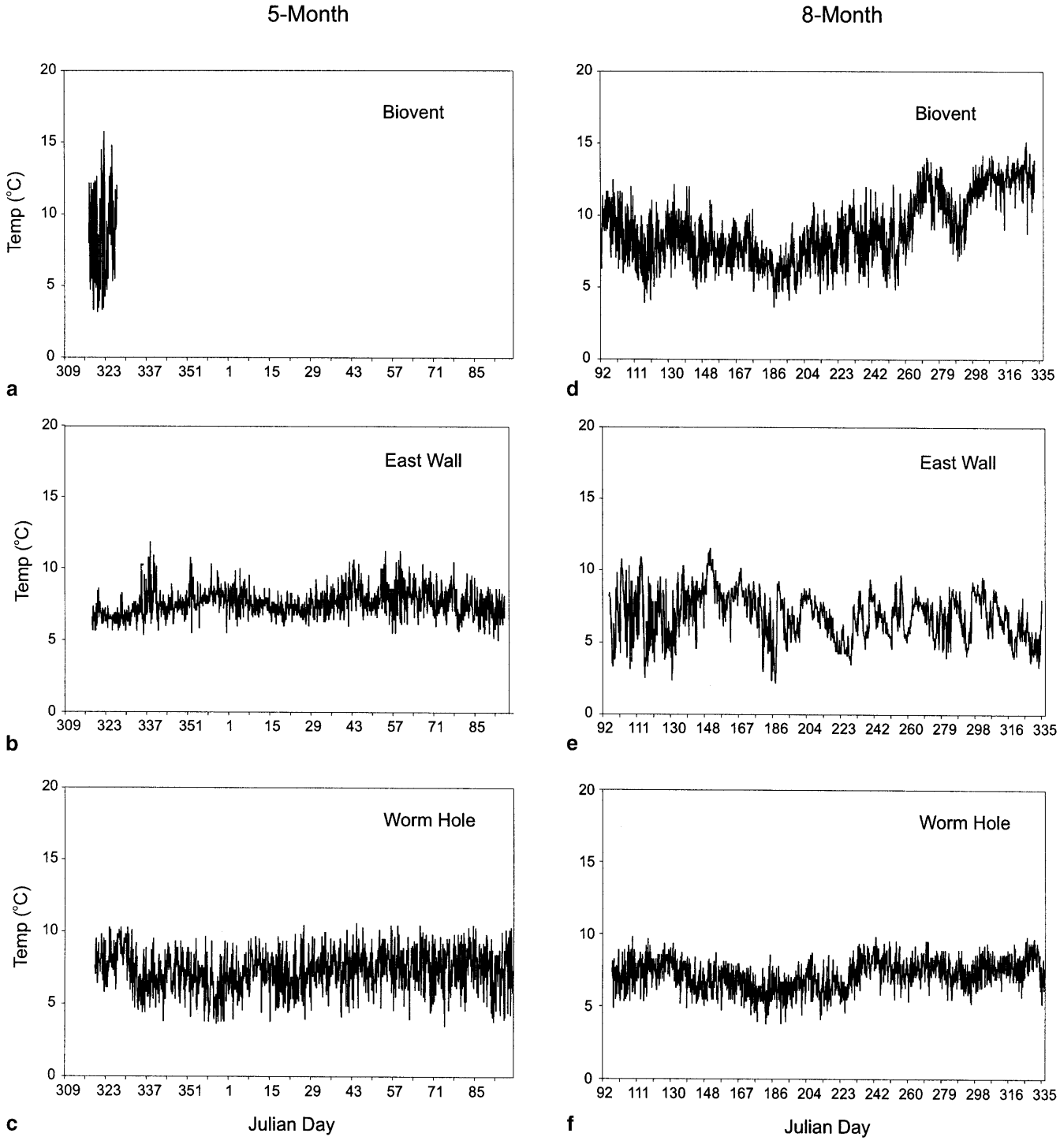

Fig. 3 Temperatures measured with internally-recording "Hobo" probes over **a-c** the 5-month (November 1994–April 1995) and **d-f** 8-month (April–December 1995) intervals at the **a, d** Biovent, **b, e** East Wall, and **c, f** Worm Hole vent sites. Measurements were typically made within the same vestimentiferan tubeworm clumps where blocks were deployed, but not at the position of any one particular block. **a** The 5-month probe at Biovent failed after 10 days. The temperature trend visible in the final 2 months of the 8-month record from Biovent was not apparent in any other probes in that same vent field, and appears to have been a local anomaly. **e** The 8-month probe at East Wall was located at a site near the specific East Wall vestimentiferan clump containing blocks (the probe nearest the blocks was not recovered)

(Fig. 4). At Worm Hole, where the early-successional species *T. jerichonana* so vastly dominated and where our basalt blocks were placed within monospecific *T. jerichonana* clumps, all three species of vestimentiferans successively colonized in substantial numbers. Similarly, at Biovent, where we never saw *T. jerichonana* present on the natural seafloor during our study and where our blocks were placed within *R. pachyptila* clumps, all three species of vestimentiferans colonized in large numbers. Finally, at East Wall, where *R. pachyptila* dominated over *T. jerichonana*, all three tubeworms colonized. Nu-

Table 1 Abundances of the three vestimentiferan species, *Tevnia jerichonana, Riftia pachyptila*, and *Oasisia alvinae*, colonizing basalt blocks at three vent sites (Biovent, East Wall, Worm Hole) over three time intervals (5, 8 and 13 months). Surrounding fauna is characterized as abundant *R. pachyptila* (*Rif-A*), mixed *R. pachyptila* and mussels (*Rif-M*), sparse *R. pachyptila* (*Rif-S*), abundant *T. jerichonana* (*Tev-A*) or sparse *T. jerichonana* (*Tev-S*). Most blocks were uncaged (*Unc*), but some were suspended in mesh cages (*Cag*), or in cage-controls lacking mesh on one side (*Con*). Temperature anomaly represents the maximum anomaly from ambient (1.8°C) recorded at the base of each block or cage when it was placed into (*in*) and removed from (*out*) the habitat. Vestimentiferans were identified to species via molecular (*Mol*) techniques, or were unidentified (*Unk*). Only blocks recovered from a suitable microenvironment (defined as where the recorded temperature anomalies exceeded 2°C or where healthy vestimentiferans surrounded the block) were included

Site	Interval (months)	Fauna	Cage	Temperature anomaly		Vestimentiferans				
				In (°C)	Out (°C)	*Tevnia*	*Riftia*	*Oasisia*	Unk	Total
Biovent	5	Rif-M	Unc	2.8	0.4	8	5	7	20	40
	5	Rif-M	Unc	13.3	NR	6	4	0	42	52
	5	Rif-M	Cag	13.1	NR	0	0	0	0	0
	5	Rif-M	Cag	7.4	22.7	0	0	0	0	0
	5	Rif-S	Cag	2.4	10.2	0	0	0	0	0
	5	Rif-M	Unc	13.2	3.0	–	–	–	4	4
	5	Rif-M	Unc	2.5	NR	–	–	–	4	4
	5	Rif-S	Unc	2.9	12.0	0	0	0	0	0
	8	Rif-M	Unc	NR	6.8	–	–	–	85	85
	8	Rif-M	Unc	NR	6.2	11	1	4	131	147
	13	Rif-A	Cag	13.0	3.7	2	0	6	265	273
	13	Rif-A	Cag	11.5	8.0	1	4	6	333	344
	13	Rif-A	Unc	10.7	2.8	6	2	2	105	115
	13	Rif-A	Unc	13.8	3.7	–	–	–	291	302
	13	Rif-M	Unc	12.3	8.0	–	–	–	227	227
	13	Rif-M	Unc	1.0	2.8	–	–	–	41	41
East Wall	5	Rif-A	Unc	13.2	5.2	24	14	10	355	403
	5	Rif-A	Cag	1.7	6.2	0	0	0	0	0
	5	Rif-S	Cag	3.7	5.2	0	0	0	0	0
	5	Rif-A	Cag	6.2	3.2	0	0	0	0	0
	5	Rif-A	Unc	2.2	6.2	0	0	0	0	0
	5	Rif-A	Unc	3.4	3.2	0	0	0	0	0
	8	Rif-A	Cag	6.2	3.5	19	0	0	13	32
	8	Rif-A	Con	6.2	2.1	27	0	2	723	752
	8	Rif-A	Con	1.2	1.4	18	0	2	58	78
	8	Rif-A	Con	5.2	NR	10	1	13	155	179
	8	Rif-A	Unc	5.2	NR	7	3	14	169	193
	8	Rif-A	Cag	5.2	NR	–	–	–	1	1
	8	Rif-A	Unc	6.2	1.5	–	–	–	3	3
	8	Rif-A	Unc	1.2	2.1	0	0	0	0	0
	13	Rif-A	Unc	10.2	1.8	25	0	0	51	76
Worm Hole	5	Tev-A	Unc	8.2	9.1	16	2	1	224	243
	5	Tev-A	Cag	9.4	5.0	0	0	0	0	0
	5	Tev-A	Cag	8.9	5.5	0	0	0	0	0
	5	Tev-S	Unc	7.9	3.5	0	0	0	0	0
	8	Tev-A	Unc	3.5	2.8	8	3	4	77	92
	8	Tev-A	Unc	8.2	7.7	3	11	3	55	72
	8	Tev-S	Unc	2.4	4.1	0	0	0	0	0
	13	Tev-A	Unc	7.1	2.9	11	1	3	716	731
	13	Tev-A	Unc	8.2	6.5	13	1	6	209	229

merically, *R. pachyptila* did not show a pattern of relative abundance among the genetically identified colonists that matched the spatial pattern among vent sites: at the vent site with lowest *R. pachyptila* abundance and biomass among the vestimentiferans observable on the natural seafloor, Worm Hole, *R. pachyptila* made up 21% of all genetically identified colonists, as compared to 19% at Biovent and 10% at East Wall, where *R. pachyptila* adults were so dominant (Table 1). Likewise, in both independent time periods, the 5-month and the 8-month intervals, all three vestimentiferan species colonized our basalt blocks in substantial numbers at every site (Fig. 4). Thus, larvae were available for colonization during all time periods at all sites for each species.

T. jerichonana demonstrated colonization responses on our blocks that are consistent with the life history of a weedy species. *T. jerichonana* was the most abundant colonist, making up 60% of all genetically identified vestimentiferans, as compared to 26% for *O. alvinae* and 14% for *R. pachyptila* (Table 1). Accordingly, *T. jerichonana* was present on all blocks colonized by identifiable vestimentiferans, whereas *R. pachyptila* and *O. alvinae* occurred only on some (Fig. 4). This result was not due to insufficient sampling of rarer species because,

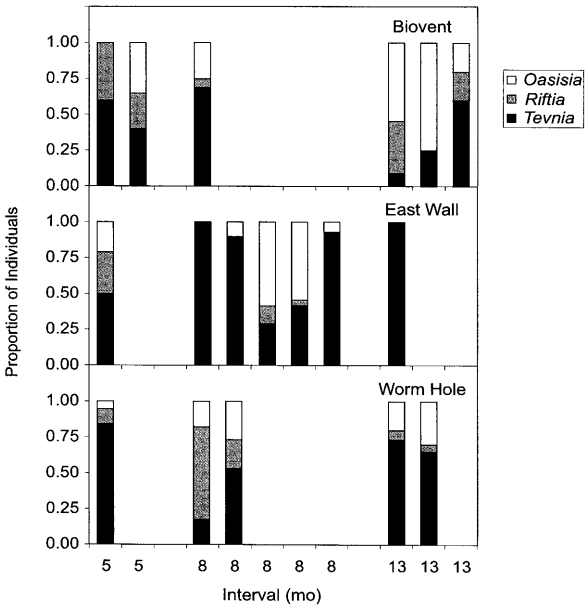

Fig. 4 Relative abundance of the three vestimentiferan species, *T. jerichonana*, *R. pachyptila*, and *O. alvinae* on basalt blocks recovered after 5-month, 8-month and 13-month intervals at the Biovent, East Wall, and Worm Hole vent sites. Only blocks with at least one genetically identified vestimentiferan colonist are shown. Total numbers of identified colonists on each block are given in Table 1

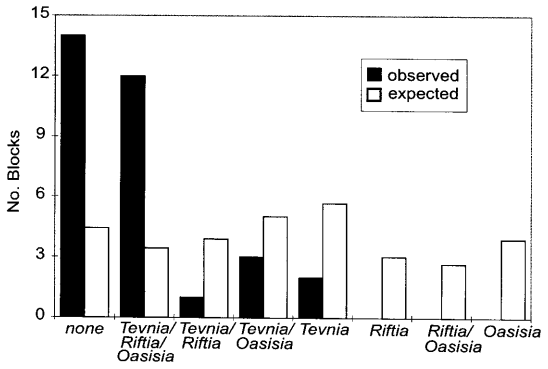

Fig. 5 Number of basalt blocks colonized by individual vestimentiferan species, *T. jerichonana*, *R. pachyptila*, and *O. alvinae*, or combinations of species. Blocks were recovered after 5-month, 8-month and 13-month intervals at the Biovent, East Wall, and Worm Hole vent sites. Only blocks with at least one genetically identified vestimentiferan colonist are shown

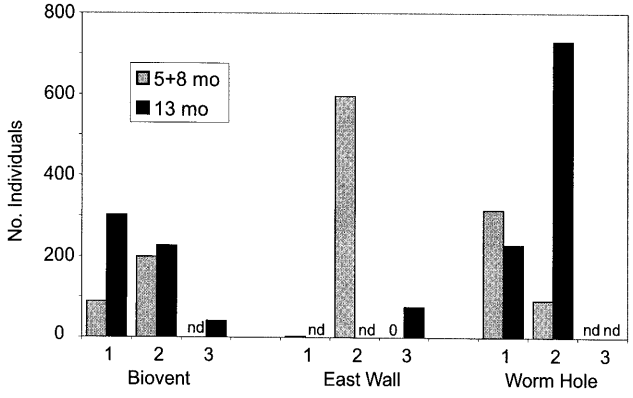

Fig. 6 Number of vestimentiferan colonists summed from basalt blocks deployed for 5- and 8-month intervals and compared to blocks deployed for 13-month intervals in the same replicate tubeworm clump. Loss of 1 block from the 8-month interval at Biovent, 2 blocks from the 13-month interval at East Wall, and 1 block each from the 5-month and 13 month intervals at Worm Hole resulted in no data (*nd*); other blocks had no colonists (*0*)

when *R. pachyptila* and *O. alvinae* did occur, they did so in substantial numbers. *R. pachyptila* and *O. alvinae* were never detected in the absence of *T. jerichonana* on our blocks, although *T. jerichonana* did occur alone (Fig. 5). The pattern of colonization of blocks differs significantly (χ^2=57.2, n=8, P<0.01) from what would be expected if each species colonized blocks independently and with a probability equal to their observed frequency of occupation of blocks. Blocks occupied by all three species of vestimentiferans and totally unoccupied blocks were more than twice as frequent as expected, whereas blocks occupied by *R. pachyptila* and/or *O. alvinae* in the absence of *T. jerichonana* were never observed, despite the expectation that they would constitute 30% of all blocks (Fig. 5).

Our further tests of independence in the colonization process involved contrasts over time of total numbers of colonizing vestimentiferans. These contrasts utilized the complete counts of all vestimentiferan colonists on uncaged blocks independent of species (our limited identifications prevent an analogous analysis by species). The number of vestimentiferan colonists on blocks from the continuous 13-month interval (mean=313 individuals per block) tended to be higher than the summed numbers from the 5-month and 8-month blocks (mean=128 individuals per block) from the corresponding replicate tubeworm clumps (Fig. 6). To control for likely and apparent effects of clump, we conducted a paired *t*-test with pairing based on clump of deployment. The loss of several critical blocks from the 8-month and 13-month intervals reduced the replicates for this analysis, and the pattern of higher colonization over the continuous 13-month interval than predicted by the sum of the 5- and 8-month intervals was not significant (two-sample, paired *t*-test: t=–1.45; n=5; P=0.22).

Discussion

We offered three initial hypotheses for why *T. jerichonana* precedes other vestimentiferan species at vents but is eventually succeeded by *R. pachyptila*. The physical/chemical hypothesis presumes that the succession of species is driven by progressive changes in the fluid flux at a given vent site and differential preferences or tolerances of the species. We found no correlation between the species composition of colonizing vestimentiferans and temperature (which serves as a proxy for chemical environment) among blocks (Fig. 2), indicating that

these vestimentiferan species do not have significantly different physical/chemical preferences or tolerances at this stage of their life history. Furthermore, the physical/chemical hypothesis predicts that a pioneer species would tend not to colonize a habitat in a later successional stage. This was clearly not the case on the spatial scale of tubeworm clumps, as *T. jerichonana* was a frequent colonist of blocks inside clumps of *R. pachyptila* at both East Wall and Biovent (Table 1). Thus, our data provide no support for the physical/chemical explanation for observed patterns (Shank et al. 1998) of succession from *T. jerichonana* to *R. pachyptila* at the East Pacific Rise.

The larval dispersal hypothesis predicts that patterns of colonization of each species would depend on the availability of larvae to settle from the plankton. If there were a period when *R. pachyptila* or *O. alvinae* larvae were not present in the overlying water column (or present but not competent to settle), blocks missing either of these species would all occur in the same time interval. Similarly, if *R. pachyptila* or *O. alvinae* larvae were limited in the spatial extent of their dispersal capabilities, then we would expect the blocks missing these species to occur in a specific location. Contrary to these expectations, blocks missing *R. pachyptila* or *O. alvinae* recruits co-occurred with blocks colonized by these species in all three sites in both the initial 5-month and subsequent 8-month time intervals (Fig. 4). Because *R. pachyptila* and *O. alvinae* recruits were present in substantial numbers on at least some blocks in all sites during all time intervals, their absence on other blocks cannot be attributed to poor dispersal or colonization abilities on the temporal and spatial scales involved in our experiment. Interestingly, the relative proportion of *R. pachyptila* recruits among genetically identified vestimentiferans was not lower at the Worm Hole site (which was dominated by adult *T. jerichonana*) than at the Biovent and East Wall sites (dominated by adult *R. pachyptila*). This observation suggests that species composition of the locally established vestimentiferan population is not necessarily a good predictor of future colonists to unoccupied surfaces. This lack of provincialism on scales of kilometers is not surprising given that tidal flows along this section of the ridge are strong enough to transport larvae as far as 2 km in a single tidal excursion (Kim and Mullineaux 1998).

The facilitation/competition hypothesis predicts that colonization by a later-succession species depends on (in the obligate version) or is enhanced by (in the facultative version) the presence of precursor species and is not purely a function of the chemical environment or availability of larvae. Several of our results are consistent with this hypothesis: (1) the observation (Fig. 4) that *R. pachyptila* and *O. alvinae* occupied only those blocks that also held *T. jerichonana*; (2) the demonstration (Fig. 4) that *T. jerichonana* was able to colonize empty space on blocks placed in habitats that supported adult *R. pachyptila*; (3) the pattern of non-random distribution of the three vestimentiferan species, in which blocks colonized by all three species and blocks remaining uncolonized were far more abundant than expected while blocks containing *R. pachyptila* and/or *O. alvinae* without *T. jerichonana* were non-existent (Fig. 5); and (4) the non-significant trend towards facilitation of abundance of colonists by prior arrivals, as reflected in greater densities of colonists over 13 months than for the sum of the component 5- and 8-month intervals (Fig. 6). Because of its fast growth rate and larger adult size (Lutz et al. 1994), it seems reasonable to imagine that *R. pachyptila* would be competitively superior to *T. jerichonana*.

The failure of the three vestimentiferan species to occupy blocks independently, based on their observed frequencies of colonization (Fig. 5), deserves some careful scrutiny and consideration. Three types of departures from expectation drive the significance. First, more blocks go completely uncolonized than expected by chance alone. Such a response does not appear to have been due to placement of those blocks in unsuitable physical/chemical environments. We removed from analysis all uncolonized blocks with maximum temperatures that fell below a 2°C anomaly and all blocks not placed directly within and abutting clumps of thriving adult vestimentiferans. Second, the number of blocks colonized by all three species was far larger than expected. This could be driven by some mutual facilitation of settlement. If so, that could also explain the trend towards enhanced numbers of vestimentiferan colonists over 13 months as compared to the sum of the component 5- and 8-month intervals. Interspecific facilitation of settlement was clearly not required for *T. jerichonana*, which alone among the species occupied blocks that contained no other species of vestimentiferan (Fig. 4). The third contributor to the pattern of departure from random expectation in the colonization patterns of blocks was this absence of blocks containing *R. pachyptila* and/or *O. alvinae* without *T. jerichonana*. Thus, the facilitation is one that appears to affect not the pioneer species, *T. jerichonana*, but instead the other two vestimentiferans.

The mechanism by which *T. jerichonana* could facilitate colonization by subsequent vestimentiferan species is not resolved. Positive interactions have long been recognized as an important aspect of community structure and temporal change by plant ecologists and by rocky intertidal ecologists (e.g., Clements 1916; Connell and Slatyer 1977; Bertness and Leonard 1997). Theoretical considerations suggest that positive interactions among species should be more prevalent in physically stressed environments (Bertness and Callaway 1994). Vents are characterized by potentially toxic effluent with strong gradients and unpredictable fluctuations, and they are typically thought of as being stressful. In physically stressed plant communities, however, the facilitating species mitigates the stress for subsequent colonists, by processes such as reducing evaporation, providing physical shelter from wind, sun, or waves, or reducing predation. Small, newly settled individuals of *T. jerichonana* are unlikely to provide any analogous modification of the physical environment. They may conceivably pro-

vide some chemical protection for their associates from predation (Hay 1986). However, the most likely process by which *T. jerichonana* facilitates settlement of other vestimentiferans is through provision of a chemical cue for settlement either directly (as in Pawlik 1992; Zimmer-Faust and Tamburri 1994) or by modifying the local bacterial populations. There is abundant and growing evidence for the importance of larval habitat selection in marine invertebrates (Pawlik and Butman 1993; Tamburri et al. 1996; Orlov 1997; Wieczorek and Todd 1997). Each of these scenarios is consistent with our qualitative observations that newly settled vestimentiferans typically occur in aggregations on scales of centimeters. As in many plant communities, it is likely that the facilitation in vestimentiferan species is not obligate. *R. pachyptila* and *T. jerichonana* co-occur along the northern (Tunnicliffe et al. 1998) and southern East Pacific Rise, but *T. jerichonana* has not been reported from Guaymas Basin or the Galapagos Rift. It is not clear whether its absence in these locations is due to a restricted biogeographic range, incomplete sampling, or the advanced successional stage of those communities.

The use of biogenic cues for habitat selection by marine invertebrate larvae or any dispersing organism capable of choosing its subsequent life-long location represents an alternative to use of physical/chemical information on habitat quality. Even in the absence of any possible environmental ameliorization provided by *T. jerichonana* and its tubes, there is a logical basis to expect selection to favor use of biogenic cues for settlement by later-succession vestimentiferans. The physical/chemical environment of deep-sea vents has been shown to experience dramatic and unpredictable fluctuations on daily to decadal time scales in the flux of venting fluids (Fig. 3; Fornari et al. 1998), thereby influencing most critically important aspects of the habitat, such as temperature, H_2S and metals concentrations, and resulting microbial production (food supply). Consequently, the use of the instantaneous state of a physical/chemical variable as a measure of environmental suitability by a dispersing propagule such as a larval invertebrate to select adult habitat entails a high potential for fatal error. On the other hand, the very presence of weedy earlier-successional species implies that a favorable physical/chemical environment has persisted at that location for some period of time, at least as long as the weeds are old. Thus, use of a biogenic cue for habitat selection produced by weedy predecessors has the effect of integrating environmental conditions over some substantially longer period of time than is ever possible from making instantaneous measurements of physical/chemical conditions. Such use of biogenic cues should be especially common among later-succession species that inhabit environments characterized by extreme and unpredictable physical environmental variation on time scales shorter than the generation times of the organisms. Although the deep-sea vent system represents one example of such a system, we have not yet determined whether this is the mechanism acting to induce facilitation among vestimentiferan tubeworms. Nevertheless, this process represents a reasonable possibility suggested by our data and the concept should find application to numerous other ecological communities on land and in water, thus representing an intellectual legacy of ecological research on vents.

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