CHEMOSYNTHETIC COMMUNITIES IN THE DEEP SEA: ECOLOGICAL STUDIES

by

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Submitted to the Department of Biology
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Abstract

Deep-sea benthic communities dependent on chemosynthetic primary production are associated with areas of active venting of chemically-modified seawater. Patterns in the distribution of species that occur at hydrothermal vents can be used to predict locations of the vent sites. Patterns in the distributions of species among vents along ridge segments are used to identify the spatial scales over which biological and physical processes operate to control community composition. Within a vent, a zonation in species distributions correlates with gradients of temperature and water chemistry. Along a given ridge segment, vent communities share the same species pool, but the relative abundance of each species varies from one site to another. On a basin-wide scale, the fauna of vent communities represent biological continua, where gradual morphological and genetic differentiation in species is correlated with increasing distance between vent sites. Differentiation of distinctive faunal assemblages at vents occurs at a global scale. Populations of species at vents are established and maintained through recruitment of larval stages. To study recruitment processes at vent sites, slate panels were placed at and near vent sites on the seafloor for varying lengths of time. Size distributions of animals on retrieved panels suggest that recruitment is an intermittent or continuous process rather than a single episodic event. Recruitment of vent-associated species was greater on panels placed within vent communities compared to panels placed adjacent to these communities, a pattern consistent with the observed maintenance of communities in discrete regions of hydrothermal flux.

The trophic structure of chemosynthetic communities can be complex. Primary production by chemoautotrophic bacteria can take place within host tissues of some invertebrates as well as on surfaces and in the water column and subsurface conduits. Carbon and nitrogen isotopic compositions of host tissues can be used to demonstrate the dependence of symbiont species on chemosynthetically-derived organic material. From the patterns in the isotopic compositions of vent and seep symbionts, potential sources of inorganic carbon are identified. Deep-water dissolved inorganic carbon serves as a large, isotopically buffered pool of inorganic carbon used by tubeworms and bivalves at hydrothermal communities of Juan de Fuca, Gorda, Guaymas Basin, East Pacific Rise, Galapagos, and Marianas vents. Variability in tubeworm carbon isotopic compositions at seeps may be attributed to significant

contributions of isotopically variable DIC in seep effluents. Isotopic techniques are also used to explore trophic relationships among a variety of heterotrophic and symbiont-containing fauna at Hanging Gardens on the East Pacific Rise and at Marianas vents. Carbon isotopic measurements suggest that free-living bacteria are important sources of food at both sites. Nitrogen isotopic analyses show that the Marianas community may be simpler in trophic structure than the Hanging Gardens community. The biomass of most known vent sites is conspicuously dominated by large invertebrates with symbiotic bacteria. At vent sites on the Mid-Atlantic Ridge, large swarms of shrimp dominate the biomass. There is no evidence for endosymbionts in these shrimp, based on analyses of morphology, stable isotopes, lipopolysaccharides and ribulose-1,5-bisphosphate carboxylase activity. Instead, the shrimp appear to be normal heterotrophs, grazing on free-living microorganisms associated with black smoker chimneys. High bacterial productivity within the sulfide matrix of the chimneys must be required to sustain the shrimp populations.

Hydrothermal vent environments exhibit some of the most extreme gradients of temperature and chemistry found in the biosphere. Many of the animals that colonize vent sites exhibit adaptations that allow them to exist in such an unusual environment. A novel eye in shrimp from Mid-Atlantic Ridge vents is described. The eye, comprised of a pair of large organs within the cephalothorax, contains a visual pigment but lacks image-forming optics. The eye appears to be adapted for detection of low-level illumination and ~~are~~ is suggested to have evolved in response to a source of radiation associated with the environment of hydrothermal vents. An electronic camera was used to detect light emitted from high-temperature (350°C) plumes that rise from the orifice of black smoker chimneys on the Endeavour Segment of the Juan de Fuca Ridge. Calculations suggest that thermal radiation from hot water may account for most of the light detected and that this light may be sufficient for geothermally-drive photosynthesis by bacteria.

Thesis Supervisor: Dr. J. Frederick Grassle, Senior Scientist, Woods Hole Oceanographic Institution

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Biography

Cindy Lee Van Dover was born in Red Bank, New Jersey on 16 May 1954. In 1972, Ms Van Dover joined the first class of women to enter Rutgers College and in the summer of 1973, Ms. Van Dover became the first woman to work at the Rutgers University Shellfish Research Laboratory in Green Creek, NJ, a laboratory that has been in operation for over 100 years. Throughout her tenure as an undergraduate student, Ms Van Dover continued her employment with the Shellfish Laboratory, excepting a 9 month leave of absence in 1975-1976 to participate as a Fellowship Student at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.

On receiving her B.S. degree, Ms Van Dover ventured south to North Carolina where she worked first with a fish biologist at the University of North Carolina's Institute of Marine Science in Morehead City and then with an invertebrate zoologist at the Duke University Marine Laboratory in Beaufort.

A 4 month back-packing trip in Maine during the fall of 1978 followed by a hitch-hiking trek from Maine to Florida culminated in employment at the Smithsonian Institution's Ft. Pierce Bureau (Ft. Pierce, Florida), where Ms Van Dover worked as a translator (Russian to English) and a student of larval crustacean biology. She also found employment at the Florida Medical Entomological Laboratory (Vero Beach, FL) studying the ecology of pitcher-plant and tree-hole dwelling mosquitoes.

A nine-month period of unemployment in Ithaca, NY during 1981-1982 ended with the author's participation in the OASIS expedition to hydrothermal vents at 21°N on the East Pacific Rise and her first exposure to deep-sea research and submersible operations. Following this work, Ms Van Dover began graduate studies in the biology department of the University of California, Los Angeles, where she received a Master's Degree in 1985. One of her accomplishments during this period was to finish and place in the 1985 Castaic Triathalon. Also during 1985, Ms Van Dover saw her first view of the deep-sea floor through the portholes of ALVIN while participating in the Galapagos '85 Expedition.

From UCLA, Ms Van Dover returned to Woods Hole, MA to work as a technician at the Marine Biological Laboratory, analyzing material collected from hydrothermal vents.

In June 1986, Ms Van Dover entered the Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography where she completed the work presented in this dissertation and defended on her 35th birthday.

PREFACE

"... if nature still holds secrets for us ... nothing is more reasonable than to admit the existence of ... new species or types living in a special environment at the bottom of the sea."

Monsieur Arronax
in Jules Vernes'
20,000 Leagues Beneath the Sea

Monsieur Arronax prophesied well. At depths from a few hundreds of meters to thousands of meters, productive communities of benthic invertebrates colonize cold seeps and hot vents on the seafloor. These communities are energetically dependent on microbial chemosynthetic oxidation of reduced chemical species (such as H_2S and CH_4) in which seep and vent fluids are enriched. Over 100 new species of invertebrates, belonging to more than 50 new genera, 25 new families or subfamilies, and 1 new phylum, have been collected from seeps and vents. Within this gallery of recently-described organisms are species that support new types of symbioses between invertebrates and chemoautotrophic bacteria.

Discovery of deep-sea vent communities at the Galapagos Spreading Center in 1977 created a new frontier in biological oceanography. The exotic and unexpected plethora of life at vents raised a series of fundamental biological questions: What energetic substrates support the biological activity? How is energy transferred through the system? What biotic adaptations are required to sustain life at vents or seeps? How are populations maintained and altered over time? What patterns

exist in species composition and spatial distribution within and among communities, and what environmental and biological processes determine such patterns? These questions and the anticipation of adventure and discovery motivated the research documented in this dissertation.

The chapters that follow are loosely organized into three principal themes: Faunal Distributions, Trophic Relationships, Vision and Light.

Four different approaches to the study of faunal distributions were undertaken. The first approach (Chapter One) is the description of the distribution of a single species - the brachyuran crab *Bythograea thermydron* - in relation to vent sites along a ridge axis. Chapter Two is a community approach wherein the distribution of dominant vent invertebrate species are reviewed at three different spatial scales, namely i) within vent sites; ii) among vents along a segment of ridge axis; iii) among ridge segments. Chapter Three celebrates the discovery of vent fields on Gorda Ridge and places the faunal composition of Gorda vent communities within a regional context. An experimental approach to faunal distributions by documentation of recruitment events at and near vent communities is described in Chapter Four.

Four chapters are devoted to studies of trophic relationships using stable isotopic techniques. In Chapter Five, a review of the literature relating to isotopic studies of vent and seep fauna is presented. These studies focus on host-endosymbiont relationships and sources of organic and inorganic carbon within chemosynthetic communities. New data on isotopic compositions of symbiont species from vent and seep environments are presented in Chapter Six and patterns in isotopic

compositions of symbionts are identified and interpreted. Chapter Seven examines the feeding biology of the shrimp, *Rimicaris exoculata*, that occurs in vast numbers on black smoker chimneys at Mid-Atlantic Ridge vent sites. The methods used to determine the diet of the shrimp include isotopic techniques along with gut content analyses and functional morphology. A community-level approach to the study of trophic relationships at vents using isotopic techniques is explored in Chapter Eight.

Two chapters examine vision and light at vents. In Chapter Nine, a novel visual organ in shrimp from Mid-Atlantic Ridge vents is described and documented and a stimulus of visible light from 350°C black body radiation is proposed. Chapter Ten follows with a description of light emitted from a 350°C black smoker and presents the thesis that the potential of geothermally-driven photosynthesis at vents cannot be dismissed.

In an appendix, a final chapter describing the zooplankton community overlying the Guaymas Basin hydrothermal vent fields is presented.

PART I

FAUNAL DISTRIBUTION STUDIES

Chapter 1

Limnol. Oceanogr., 32(4), 1987, 1006–1010

Prediction of hydrothermal vent locations from distributions of brachyuran crabs[1,2]

Abstract—We apply a model of symmetrical, exponential decreases in brachyuran crab density with distance from vent centers to a video census of crab density along the neovolcanic zone of the East Pacific Rise to predict the number of vents and their locations. Our model, calibrated by data from two known vents, accurately locates one other known vent and predicts the locations of three additional vents presumably skirted by the towed camera sled. Analysis of crab distributions may thus be a valuable tool for mapping the locations of hydrothermal vents with remotely operated camera gear.

Mapping the distribution of hydrothermal vents along a spreading center is prerequisite to developing models of oceanic heat budgets or hydrological and geochemical balances. Models of biological processes, such as colonization and maintenance of hydrothermal vent communities, also rely on knowledge of spatial relationships among vents. Photographic surveys, with towed camera arrays, are increasingly used to map geological features of midoceanic spreading centers and locations of hydrothermal vents. Because vent fields are typically small and camera images cover only narrow swaths of the seafloor, it is possible to pass very close to vents without seeing them. Lichtman et al. (1984) used increased densities of nonvent, megafaunal invertebrates as indicators of proximity to vent sides. Their approach, based on the number of benthic organisms counted on 10 successive exposures, is qualitative and does not predict coordinates of vent locations. We provide here a statistical model, based on distribution patterns of the brachyuran crab *Bythograea* and constrained by parameters derived from transects across known vent areas, that predicts both the number and locations of hydrothermal vents along ridge axes. *Bythograea* is known to occur at hydrothermal vents; we provide the first quantitative data documenting its distribution on nonvent terrain using density estimates derived from videotapes of the seafloor recorded by the ARGO camera system.

ARGO camera gear and navigation techniques are described elsewhere (Harris and Ballard 1986). The ARGO-24 lowering of the ARGORISE Expedition (December 1985) produced 22 h of nearly continuous ½-in. (1.3 cm) video coverage of the seafloor along the axial ridge of the East Pacific Rise from 10°49′N to 10°43′N. This portion of the ridge lies at 2,600 m on a shoulder midway between the regional topographic high (2,550 m) to the north (10°55′N) and the low (3,800 m) at the Clipperton Fracture Zone to the south (10°15′N) (MacDonald et al. 1984). In the northern portion of the transect, the seafloor is predominantly old pillow and lobate lava flows; to the south,

[1] This research was supported by ONR contract N0014-82-C-0019 and Navy Chair grant N0014-85-G-0242 to R.D.B., NSF grant OCE 83-11201 to J. F. Grassle, and the Woods Hole Oceanographic Institution Education Office. C.L.V.D. was supported in part by a National Science Foundation Graduate Fellowship.

[2] Woods Hole Oceanographic Institution Contribution 6331.

beginning at 10°46′N, the terrain is dominated by fresh, glassy, lobate flows and associated collapse features.

Recognition of crabs on the videotapes and their identification to the family Bythograeidae was facilitated by ARGO-24 still photographs that included one or more crabs. Densities of brachyuran crabs along the ARGO-24 track were determined by reviewing videotapes and maintaining a running tally of the number of crabs observed over 5-min intervals, taking into account overlap in the field of view when the image switched between forward- and downward-looking cameras. Altitude of ARGO video cameras was variable, primarily in response to surge of the surface ship. Records of crab densities were made only when the camera was < 12 m off the bottom; above this height, resolution of crabs was not reliable. Average altitude during 5-min intervals (calculated from altitudes recorded every 20 s) was 7.8 ± 1.3 m. Width of the swath covered by the video image = 1.6 × altitude; thus an estimate of the width of the area analyzed is 12.4 ± 2.1 m. We do not correct for altitude since, even over 20-s intervals, camera altitude varied by several meters. The camera track was 18 km long, meandering southward along the spreading center from 10°49′N to 10°43′N (linear distance, 12 km). Latitude (x) and longitude (y) corresponding to the location of the camera sled at the start of each 5-min interval were determined from the ARGO navigation data base.

Three vents were observed along the transect; these known vents are referred to as K1, K2, and K3, numbering from north to south. Unknown vents predicted by the model are labeled U1, U2, etc., again numbering from north to south.

Crab densities along transects approximately perpendicular to the spreading axis at vents K1 and K2 at 10°45.0′N and 10°46.6′N are illustrated in Fig. 1. The vents themselves appeared to be of the low-temperature, Galapagos-type, with water emanating between lobes of lava. The associated megafauna was unusual, consisting of brachyuran crabs and thousands of ophiuroids colonizing the tops of the lobes. A third vent (K3) with more typical mega-

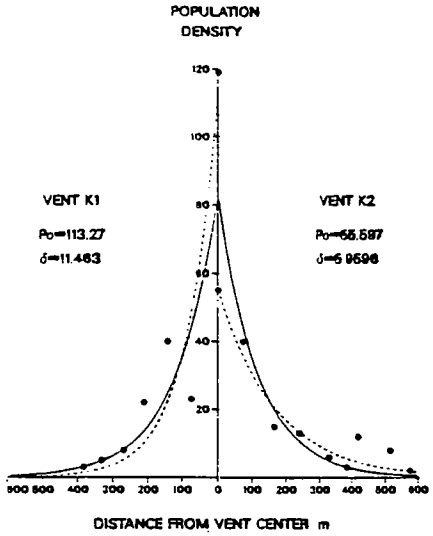

Fig. 1. *Bythograea* density (expressed as number of crabs observed during 5-min intervals along the video survey) as a function of distance from the center of a vent. Dashed lines are best fits of data from vents K1 or K2 to Eq. 1; solid lines are best fit of combined data from K1 and K2 to Eq. 1. For K1 + K2, $P_o = 83.061$ and $\delta = 8.56$.

fauna, including clams, mussels, and serpulid worms, was encountered at 10°47.7′N but was not crossed by an extended transect perpendicular to the axial valley. Crab population densities were fit to the exponential function

$$P(r) = P_o \exp(-\delta r) \qquad (1)$$

where $P(r)$ is the crab density at radius r from the vent, P_o is crab density at the center of the vent, and δ is rate of decay of crab density with distance from the vent. The parameters P_o and δ were obtained through a Gauss-Newton, nonlinear, least-squares fit of Eq. 1 to data from the two transects. Values of P_o and δ were also calculated for crab populations along each transect separately.

To predict the locations (x_i, y_i) of n vents, we used the following model:

$$P(x, y) = P_o \sum_{i=1}^{n} \exp\{-\delta[(x - x_i)^2 + (y - y_i)^2]^{1/2}\}. \qquad (2)$$

In this case, the unknowns are x_i (lat) and y_i (long), the location of the ith vent, and n, the number of vents. This model assumes

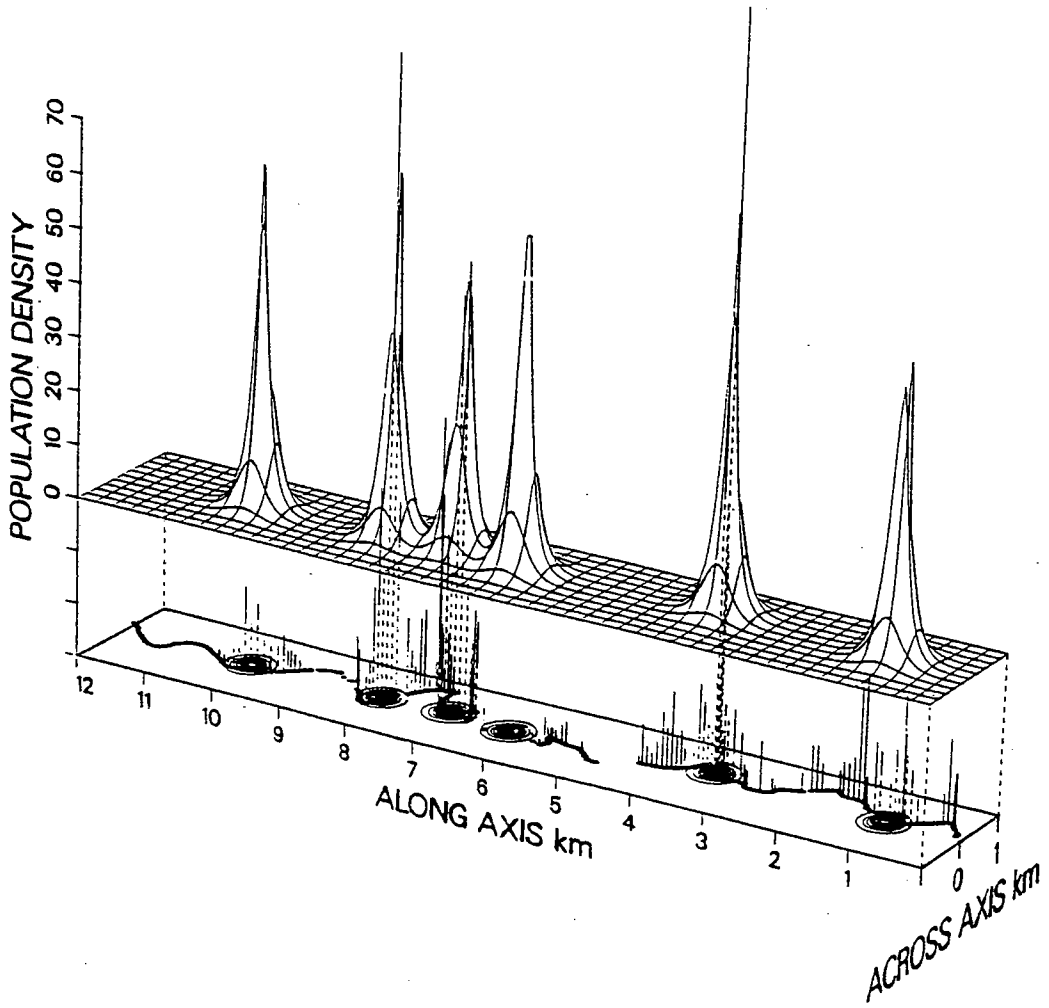

Fig. 3. Model of crab distributions along East Pacific Rise (10°49'N–10°43'N) superimposed on the survey track and actual crab density data. Three-dimensional representation of K1 + K2-calibrated model of axisymmetric exponential crab distributions. Dots marking the video track locate the start of each 5-min interval; blank spaces along the dotted track represent areas where no video data were recorded. Vertical lines—cumulative number of crabs observed along each 5-min interval; dashed portions lie under the envelope of model distributions, solid portions lie above the envelope. Crab densities were zero where track line is plotted but no vertical lines are present. Concentric rings contour the modeled crab distributions (at five-crab density intervals) onto a planar surface.

home in on vents (Spiess et al. 1980; Laubier and Desbruyeres 1985). In addition, this type of analysis may be applied usefully in marine or terrestrial systems to locate otherwise obscure or difficult-to-sample point sources, as in the identification of pollutant sources based on contaminant levels in plant or animal tissues.

We thank the personnel of the Deep Submergence Laboratory (Woods Hole Oceanographic Institution), the scientific party of the ARGORISE Expedition, and the Master and crew of the RV *Melville,* all of whom made this study possible. We have benefited from many discussions with our colleagues, especially J. R. Cann, J. F. Grassle, R. R.

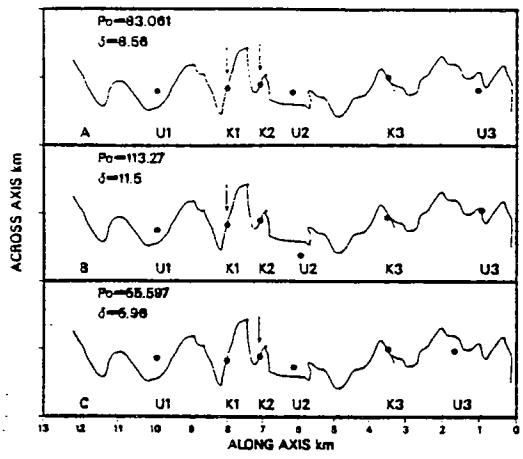

Fig. 2. Best fits of vent locations (●) with K1 + K2 (A), K1 (B), or K2 (C) calibrations applied to Eq. 2 as described in text. Wavy lines—camera track; arrows—vent(s) used to calibrate the model.

that crab distributions around vents are radially symmetric, and that every vent has the same peak population, P_o, and decay constant, δ. Sensitivity of the model to choices of P_o and δ is discussed below. More complicated models including asymmetric distributions and vent-dependent P_o and δ could easily be formulated. Our data were not sufficient to calibrate such models and thus we did not investigate sensitivity to variable P_o and δ.

Equation 2 was fitted to unweighted crab density data, $P(x, y)$, along the entire 18-km camera track with a Gauss-Newton, nonlinear, least-squares regression and the P_o and δ calculated from Eq. 1. The optimal regression was chosen according to the Akaike criterion (AIC) (Akaike 1974) where

$$\mathrm{AIC} = N \ln(\mathrm{RSS}) + 2(2n) \qquad (3)$$

in which N is the number of data points, RSS is the residual sum of squares, and $2n$ is the number of free parameters (two per vent: x_i and y_i). The number of vents, n, that minimized AIC was deemed to give the best fit of the model to the data. Figure 2 shows the best fits of vent locations with either vent K1, K2, or K1 + K2 used for calibration. For the range of P_o and δ tested, six vents consistently gave the lowest AIC, and their locations were relatively constant. The locations of the three known vents (two used for calibration, plus one other) did not change with different P_o and δ. The locations of three "unknown" vents (U1, U2, U3) varied slightly with different P_o and δ, the largest displacement being ~600 m along the N–S axis for vent U3. The lowest residual was obtained when two vents were used for calibration; residuals for the two one-vent calibrations were nearly identical, but considerably larger than the two-vent calibration. There are large gaps in the video survey and very few data points constraining the location of vent U2. According to the Schwarz criterion (Schwarz 1978) for objectively determining the optimal number of parameters in a nonlinear model, this vent should not be included, even though the AIC suggests it should; the AIC for a five-vent regression was only marginally larger than the AIC for the six-vent fit.

In Fig. 3, the best fit of the model of axisymmetric crab distributions (K1 + K2; $P_o = 83.061, \delta = 8.56$) is superimposed onto the actual data of crab abundance along the camera track. The model located vents even when the camera was towed 100–200 m away from the predicted center of a vent.

Although the existence of three vents has yet to be confirmed, the validity of the model is strengthened by the fact that it positions all of the vents along a line corresponding identically to the strike of the neovolcanic zone (Uchupi and Schwab pers. comm.). Active vents tend to occur in chains a few kilometers long, with the vents spaced 1–2 km apart, and the chains are located at, or within a few hundred meters of, the spreading axis (Francheteau and Ballard 1983; Ballard et al. 1984; Hekinian et al. 1985). In all these respects, the chain identified here is similar to other chains discovered as the result of much more intensive surveys.

The simple model described above may be a powerful and relatively inexpensive means of identifying locations of vents with active biological communities. Other organisms may be found to which a similar distribution analysis can be applied, such as the galatheid squat lobsters, *Munidopsis subsquamosa*, which form "crab gradients" that *Alvin* pilots and observers have used to

Hessler, A. Solow, C. E. S. Franks, and L. Fahrig.

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Chapter 2

Spatial Variation in Faunal Composition of Hydrothermal Vent Communities on the East Pacific Rise and Galapagos Spreading Center

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Abstract

Comparisons of distributions of megafaunal species at hydrothermal vents on the East Pacific Rise and Galapagos Spreading Center can be made at three levels: 1) within a vent field, 2) among vent fields within a cluster on a ridge segment, and 3) among ridge segments. Across forty degrees of latitude, megafaunal compositions of vent communities are remarkably consistent at the familial level. Along any given ridge segment, there appears to be a shared pool of species from which any vent field draws its fauna.

In the decade since the discovery of hydrothermal vents on the deep-sea floor, hydrothermal activity and chemosynthetically-based animal communities have been observed at many locations along major spreading axes including the Galapagos Spreading Center (GSC), the East Pacific Rise (EPR), the Juan de Fuca and Explorer Ridges, the Mid-Atlantic Ridge (MAR), and the Marianas Back-Arc Spreading Center. Comparisons of the communities at these diverse locations reveal both global similarities and individual differences. For example, a characteristic feature of nearly all known vent communities, regardless of geographical location, is the localized, concentrated biomass of living organic material. At the same time, a distinguishing feature of individual vent communities is the relative abundance of component species dominating that biomass. Not surprisingly, the greatest differences in faunal composition are seen in comparisons of dominant species between geographically disjunct spreading centers. On the EPR and GSC in the eastern Pacific Ocean, tubeworms, clams, and/or mussels typically dominate vent fields (reviewed in Grassle 1985; 1986); on the Marianas Back-Arc Spreading Center in the western Pacific Ocean, a large species of snail is predominant at vent sites (Hessler *et al.* 1988); at two known vent fields on the Mid-Atlantic Ridge in the Atlantic Ocean, a caridean shrimp prevails (Rona *et al.* 1986, Detrick *et al.* 1986, Grassle *et al.* 1986). There may even be fundamental differences in the trophic structure of vent communities: at most known vents, the dominant megafaunal invertebrate species are sessile and harbor chemoautotrophic endosymbiotic microorganisms from which the hosts presumably derive their nutrition by translocation (Cavanaugh *et al.* 1981, Felbeck, 1981, Cavanaugh 1985), but the shrimp that dominate the MAR vent

communities are highly mobile and are thought to graze directly on free-living microorganisms (Van Dover *et al.* 1988). Despite these differences in the general nature of hydrothermal vent communities on a global scale, certain elements of the vent fauna on widely-separated spreading centers may be closely related in a phylogenetic sense. Some of the best examples of this are brachyuran crabs in the genus *Bythograea*, caridean shrimp in the family Bresiliidae, and mussels in the family Mytilidae, all of which are known to occur at vents on the MAR, EPR, GSC, and Marianas Back-Arc Spreading Center. Vesicomyid clams and vestimentiferan tubeworms occur at most of these sites.

Before considering variation in faunal composition of vent communities on any scale, one needs to place vent environments within some context with respect to their spatial and chemical relationships. This context is best known for the East Pacific Rise and Galapagos Spreading Center, where the spreading axes are broken up at intervals into ridge segments by transform faults and lateral offsets up to 10's of kilometers in length (e.g., MacDonald *et al.* 1988). Most hydrothermal vent fields of the EPR and GSC explored to date occur along the shallower portions of ridge segments and are predictably arranged in short linear clusters (~10-20 km total length) along the strike of the neovolcanic zone (e.g., Ballard *et al.* 1982, Hekinian *et al.* 1983, Francheteau and Ballard 1983, ARGORISE Group, in press). South of the Gulf of California, ridge axes are exposed as bare basaltic lavas (=sediment-starved). Water depths on the order of 2400-2600 m are typical of the shallowest portion of each ridge segment, while water depths at transform faults reach 3000 m and more. The meager areal extent of individual vent fields ranges from small

pockets a few meters in diameter to fields with largest dimensions on the order of 60 to 100 m. Vent fields comprising a cluster are separated by distances of 1 to 10 km and typically range in number from 5 to 10 per cluster; distances between clusters of vent fields are less well-established, but there are perhaps 0, 1, or 2 clusters per ridge segment and therefore there may be 10's to 100's of km separating clusters (Ballard *et al.* 1982, Hekinian *et al.* 1983, Francheteau and Ballard 1983, ARGORISE Group, in press). Geological relationships among vent fields within a linear cluster are not clear. One hypothesis is that such a cluster of vent fields is the consequence of a single *en echelon* tectonic event. Biological relationships among vent fields within a cluster seem more clear-cut, with exchange of propagules among populations more likely between vent fields within a cluster than between clusters of vent fields (J.P. Grassle 1985).

Implicit in the concept of a vent field is the notion of a single hydrothermal system feeding each field. This has at least one important consequence that affects the distributions of animals within the vent community: hydrothermal circulation at one location within a vent field is likely to be strongly influenced by hydrothermal circulation elsewhere within the field. If, for example, conduits at one locale become clogged, either by inorganic precipitation and/or biologically-mediated obstruction, flow to other conduits may be increased. Alternatively, seismic activity may open up new channels, diverting flow from other locations. Presumably, the biological community will respond to modified flow rates. This response might be expressed as changes in rates of growth, reproduction, mortality, and/or colonization. Fields of dead

clams juxtaposed with vigorous populations of live clams attest to the dramatic effect modified flow rates in a low-temperature regime can have on vent communities (Hessler *et al.* 1985). The time scale for significant changes in flow rates appears to be on the order of years to 10's of years for individual vents. Fustec *et al.* (1987), for example, report changes in flow rates from one chimney and the opening of an entirely new black smoker at Actinoir on the EPR (13°N). These events occurred within a two-year time period. Campbell *et al.* (1988) cite other examples of changes in flow patterns of black smoker vents on a decadal scale of observation. Hydrothermal activity of a vent field may last longer (Lalou and Brichet 1982, 1987) and is dependent on such considerations as flow rate and time required to quench a subsurface heat source (Macdonald *et al.* 1980, Converse *et al.* 1984).

While some aspects of the geochemistry of exiting hydrothermal fluids at individual vents are more similar within a vent field than between vent fields (Bowers *et al.* 1988), two chemically distinct end-members have been observed within a single vent field of 50 m radius (Butterfield et al. 1988). Variability in the geochemistry of venting water among vent fields is expressed in the availability of reduced chemical species which in turn influences the species composition of vent communities. This is perhaps seen most dramatically in 3 vent fields at 13°N, where Bowers *et al.* (1988) report a ratio of Fe-H_2S greater than unity in end-member hydrothermal fluids. In this chemical regime, the H_2S upon which the vent fauna depends precipitates during subsurface mixing and becomes unavailable. Vent communities at these sites were absent, corresponding to the "azoic" hydrothermal fields of Ballard *et al.* (1984).

There are other important differences in the physical environment of vent fields to which the biological communities respond. For example, the type of venting also influences the species composition of vent communities (*e.g.*, Fustec *et al.* 1987). Certain species appear to be adapted to diffuse, low temperature flows emanating from cracks in the sea floor, while others appear to require the habitat associated with high temperature plumes venting from massive sulfide chimneys. In addition, local hydrodynamic regimes vary with the microtopography at each vent field. On sediment-starved ridge axes, vent communities may be located on fields of bulbous pillow lavas and flat sheet flows, on talus at the base of fault scarps, or associated with the pits formed by the collapse of ponded lavas following subsurface draining. The patterns of distributions of vent and peripheral non-vent fauna probably respond to the hydrodynamics associated with these different topographic regimes as well as to the hydrodynamics of the venting water. On sedimented ridge axes, such as the northern-most extension of the EPR including Guaymas Basin, the geochemistry of the venting water (Von Damm *et al.* 1985) and sediment (Gieskes *et al.* 1982) is distinctive, adding further dimensions with which comparisons of vent fauna on a regional scale can be made.

From these spatial and chemical relationships of vent fields and their biological consequences, there emerges a 3-tiered, spatial hierarchy that we will follow in our qualitative comparisons of vent fauna on the EPR and GSC, namely, patterns of distribution of fauna: 1) within a vent field, 2) among vent fields within a linear cluster of vent fields on a ridge segment, and 3) between ridge segments (Figure 1). Underlying this spatial hierarchy is a gradient in the probability of dispersal and

genetic exchange among populations of vent species. While we know little about these two aspects of the biology of vent fauna, they are likely to be the most important processes determining the variation in composition of vent fauna at regional and global scales. At local scales, other processes, such as recruitment, competition and predation, may control faunal composition and relative abundance of species at vent sites.

Comparisons of faunal variation within vent communities can best be made among vent fields on the East Pacific Rise and Galapagos Spreading Center. Specific fields on these ridges have been targets of repeated investigations by biologists, geologists, and geochemists. These include Guaymas Basin (27°N), 21°N, 13°N, and 20°S on the EPR, and Galapagos vents (86°W) on the GSC. In addition, there have been several large-scale photographic surveys of ridge segments and transform faults on the EPR. Documentation of vent communities on the East Pacific Rise and the Galapagos Spreading Center ranges in quality from detailed mapping of megafaunal populations and water chemistry at Rose Garden on the GSC, to video coverage of otherwise unexplored vents and single-frame snap-shots of vent animals beneath towed camera sleds on the EPR. For this reason, the comparison of vent communities that follows is restricted to a discussion of the patterns of distribution of the large, easily photographed species.

I. Distribution of Fauna within a Vent Field

i) Low temperature, diffuse flow

Our notion of a "typical" hydrothermal vent community is derived from the historical discovery of the first active deep-sea fields on the

Galapagos Spreading Center. Rose Garden is one of these fields, first visited by biologists in 1979 (Galapagos Biology Expedition Participants 1979). Subsequent discoveries and exploration of vent communities shows that Rose Garden and Galapagos vents in general are atypical in that they lack high-temperature (350°C) "blacksmoker" chimneys. Recent exploration of the EPR at 13°N (Fustec et al. 1987) and between 10°N and 12°N (Van Dover, personal observation), however, has shown that low-temperature vent fields are not uncommon along the EPR. Rose Garden does typify a generic low temperature, diffuse flow vent community, particularly in the characteristic zonation of the vent fields into three distinct habitats, each with its own suite of potentially dominant megafaunal types. Using Hessler and Smithey's (1983) original nomenclature and descriptions, these regions are designated "vent", "near-field", and "peripheral".

The warm-water "vent" region of a diffuse flow field is typically dominated by dense clusters of megafaunal species that rely to a large extent on chemoautotrophic endosymbiotic bacteria for their nutrition. These clusters are usually constrained within sharp boundaries defined by water chemistry and flow patterns of the venting water. At Rose Garden and at other diffuse flow fields on the GSC and EPR, one or another species of clam, mussel, and/or tubeworm typically dominates this region. The "vent" region is the most chemically and spatially heterogeneous environment of a diffuse flow field. Gradients of temperature and water chemistry are strongest in this region, and complex surfaces and interstices are found within the clusters of megafauna. A bythitid fish and macrofaunal populations of limpets, polychaetes, anemones, bresiliid

shrimp, lysiannassid amphipods and other small crustaceans occur within this region.

Beyond the central region are "near-field" populations of suspension-feeding and grazing megafaunal species that are presumably dependent on primary chemosynthetic production by free-living microorganisms, either directly or through short planktonic, benthic, and detrital food webs. Boundaries of these populations are sharp and again appear to be defined by gradients of water chemistry as well as by the hydrodynamic regime within the vent field. Anemones, serpulid worms (suspension-feeders), gastropods and galatheid crabs (grazers) are among the fauna that often characterize these regions.

The "peripheral" region of a diffuse flow field has the least well-defined boundaries. It is in this region that certain non-vent species may occur in greater abundance than elsewhere in the deep-sea, sometimes spectacularly so, as in the case of enteropneusts at Rose Garden in 1979. Siphonophores, deep-sea shrimp, and sponges may also contribute to the peripheral populations.

Some megafaunal vent species, notably brachyuran crabs, galatheid squat lobsters, and zoarcid fish, are not restricted to a particular region of a field. They move freely between regions and even well out into non-vent terrain. Still, they tend to be most abundant in one or another specific region.

Zonation at Rose Garden is expressed as irregular bands or rings progressing outward from the central region. This pattern of zonation is most likely due to the physical characteristics of the site: venting water flows out of a single large fissure and out of smaller collapse features

adjacent to and within a field of sheet lava. In contrast, at another diffuse flow field (21°N, EPR), venting water flows out from cracks between lobes of pillow lava, resulting in a mosaic of "near-field" fauna on the tops of the lobes surrounded by "vent" fauna in the cracks.

ii) High temperature chimneys

As noted above, vent fields on the Galapagos Spreading Center are unusual in that the hydrothermal systems are limited to diffuse low-temperature flow. At most fields on the EPR and elsewhere, hydrothermal systems include one or more massive sulfide chimneys with high temperature (200 to 350°C), turbulent plumes. These chimneys comprise a 4th habitat within a vent field. Physical and chemical gradients are at their most extreme and most complex within this habitat. Characteristic fauna associated with chimneys on the EPR and GSC are alvinellid polychaetes (3 species); brachyuran crabs, galatheid squat lobsters and zoarcid fish may also be present. Both low- and high-temperature microhabitats can occur in association with a sulfide chimney. Thus, low-temperature fauna (="vent" fauna above) can also be found on black-smoker chimneys. Not all chimneys are colonized.

II. Distribution of Fauna among Vent Fields in a Cluster of Vents.

There are 3 locations where exploration has been sufficiently detailed to provide data for comparisons at the level of vent fields within a linear cluster of vents.

i) Galapagos Vent Fields

Comparisons of megafaunal composition of 7 vent fields along an 11 km length of the Galapagos spreading axis are provided in Table 1. Data are

compiled from Corliss & Ballard (1977), Corliss et al. (1979), Crane & Ballard (1980), Hessler & Smithey (1983), Rosenblatt & Cohen (1986), and additional personal observations by R.R. Hessler. Quality of information varies among vent fields; some fields were not visited often or were more poorly photographed. Clambake 2, Dandelion Patch, and Oyster Bed were mainly documented from higher altitude photographs taken by ANGUS, making some taxa difficult to discern. Thus it is not possible to establish the absence of some taxa from some vent fields. We limit ourselves to the most conspicuous species, for which the data is most likely to be complete. More detailed comparisons await further exploration. These caveats apply to subsequent sections as well.

The three best studied fields -- Rose Garden, Clambake 1/Musselbed, and Garden of Eden -- share the full species list as far as one can tell, with two exceptions: *Neomphalus fretterae*, a limpet that colonizes hard substrates at vent openings, is absent from Rose Garden, perhaps because all the vent openings were choked with bivalves and vestimentiferans; no living *Calyptogena magnifica* were seen at Garden of Eden, but shells demonstrate past colonization.

Thus Rose Garden and Garden of Eden, which span the known length of this string of active vent fields, share a similar species pool. Yet there are obvious differences in the relative abundances of, for example, the 3 primary "vent" species -- *Riftia pachyptila*, *Bathymodiolus thermophilus*, *Calyptogena magnifica* -- among fields on this string of vents. Differences in relative abundance seem to reflect habitat differences, especially the availability of vent water, but they may be secondarily influenced by biological interactions.

A comparison of Rose Garden fauna between two visits separated by six years demonstrates that the relative abundance of taxa can change markedly with time (Hessler et al., in press). Populations of *Riftia pachyptila*, *Thermopalia taraxaca*, *Saxipendium coronatum*, anemones, and serpulids declined, while *Bathymodiolus thermophilus*, *Calyptogena magnifica*, *Munidopsis subsquamosa* and turrid gastropod populations multiplied. Hessler et al. speculate that these faunal differences are a consequence of stochastic dispersal phenomena and a decline in flow rate within the field. Thus, a single vent field can display greater differences through time than might be seen between spatially separated, contemporaneous fields.

ii) East Pacific Rise Vent Fields, 13°N

Megafaunal compositions of vent fields along a 1 km length of the EPR at 13°N (Table 2) are compiled from Desbruyeres *et al.* (1982), Laubier and Desbruyeres (1984), Fustec *et al.* (1987) and personal observations by R.R. Hessler at Pogomort and Parigo. The first two papers deal with the fauna of 13°N as a whole. Fustec *et al.* (1987) provides descriptions of the fauna of specific vent fields; Table 2 lists species' presence or absence based on this work.

In 1984, 13°N fauna was comprised of two distinct faunal assemblages, one found at high temperature vents (including *Alvinella pompejana*, *Paralvinella grasslei*, *Cyanagraea praedator*, and *Thermarces andersoni*) the other at low temperature vents (including *Riftia pachyptila*, *Tevnia jerichonana*, *Bathymodiolus* sp., serpulid polychaetes, several gastropod species, *Bythograea thermydron*, and *Thermarces andersoni*); most of the seven sites explored contained both habitats with some subset of

associated fauna (Fustec et al. 1987) although relative abundances varied from site to site. Pogomort is the primary exception, notable especially in its lack of living *Riftia pachyptila* and *Alvinella pompejana*. This field is a site of recent, former hydrothermal activity, with massive-sulfide deposits and quiescent chimneys, and with residual populations of vent fauna.

Galapagos and 13°N vent fields share many of the same species. With the exception of *Tevnia jericohnana* at 13°N, the pool of warm-water vent species is identical (although living *Calyptogena magnifica* were not observed at 13°N). Exclusively high-temperature species are obviously lacking at Galapagos vents, since that habitat is not available at the known active fields; species within this category may include *Alvinella* spp. and *Cyanagraea praedator*. Ophiuroid species and a barnacle, *Neolepas zevinae*, are added to the near-field fauna at 13°N, and the enteropneust, *Saxipendium coronatum*, is absent from peripheral regions at 13°N.

Upon detailed comparison, the singularity of two species shared between Galapagos and 13°N vent fields has been brought into question. J.P. Grassle (1985) shows that Galapagos and 13°N populations of *B. thermophilus* are electrophoretically distinct, while Jones (personal communication) notes morphological dissimilarities between populations of *R. pachyptila* at these sites. There is thus the potential for significant isolation of populations on separate ridge axes.

iii) East Pacific Rise Vent Fields, 21°N

There are at least 14 vent fields along a 30 km length of the EPR at 21°N, but the megafauna of only two of these sites is well-documented

(Table 3). Faunal composition at Clam Acres was determined from photographs and observations made on the Oasis Expedition (Hessler et al., 1985); Hanging Gardens fauna was documented on *a 1985 R/V Atlantis II* Cruise (Van Dover and C.J. Berg, personal observations).

As at Galapagos and 13°N, comparison of the fauna at vent fields along the string of vents at 21°N reveals similarities in species composition, but differences in relative abundances. *Riftia pachyptila* dominates the vent fauna at Hanging Gardens, *Calyptogena magnifica* prevails at Clam Acres; *Oasisia alvinae*, a smaller species of tube worm, is very abundant at Hanging Gardens and all but absent at Clam Acres. *Bathymodiolus thermophilus*, one of three most characteristic vent species at Galapagos and 13°N vent fields, is absent at 21°N. Serpulids and the barnacle *Neolepas zevinae* are conspicuous near-field species at Hanging Gardens, while a coiled gastropod is the most abundant near-field species at Clam Acres. Another distinguishing feature of the Hanging Gardens site is the encroachment of peripheral, non-vent species, most notably sponges (especially *Caulophacus cyanae*), into the near-field area. In contrast, no peripheral species were noted at Clam Acres. Hanging Gardens is in an unusual setting compared to other near-by vent fields, occurring along a wall of a deep and narrowing graben. A convection cell set up within the graben by the rising plume of the black smoker at one end may carry noxious vent water away from the site while bringing in a food-enriched supply of bottom water. In this way, peripheral species could encroach upon the vent habitat and thrive in a food-rich environment. Convection cells are presumed to exist at all vent sites with active hydrothermal systems (Lonsdale 1977, Enright *et al.* 1981), but the particular

topographic relief at Hanging Gardens may be well-suited to efficient flushing of vent effluents from the graben and entrapment of sinking chemosynthetic production from the plume.

Two hypotheses may be developed from these three comparisons of fauna among vent fields within clusters of vents:

1) Along any given ridge segment with a linear cluster of vent fields there exists a shared pool of species from which each individual vent field draws its fauna.

2) Relative abundances of species at a vent field, and even presence or absence of a species, are most likely functions of the field itself, with determinants being such characteristics as flow rates, water chemistry, and stochastic events (Hessler et al. 1985). The relative importance of these characteristics and the role of biological interactions in determining the species composition of vent communities remain unknown.

III. Distribution of Fauna among Ridge Segments.

At the level of comparison of vent fauna among ridge segments on the EPR and GSC, the quality of data decreases dramatically. For this reason, we focus here on distributions of a select subset of taxonomic groups, namely vestimentiferan tube worms, bivalve mollusks, and anomuran and brachyuran decapod crustaceans. These groups typically comprise the most conspicuous elements of a vent's fauna.

As noted earlier, in many locations vent communities have been photographed but not sampled, leaving taxonomic identifications to be less than perfect. Where a vent field is distant from a well-documented vent

field, we provide a generic designation from photographs with some confidence, but assume nothing about the species identification.

Data from 6 locations along the EPR and GSC are summarized in Table 4. Each of these areas is located on a different ridge segment. Sources for Galapagos, 21°N, and 13°N data have been cited above. 10-12°N and 17-20°S data were obtained by review of ANGUS photographs and/or ARGO video supplied by R.D. Ballard and from Hekinian *et al.* (1985), Renard *et al.* (1985), Kastens *et al.* (1986), McConachy *et al.* (1986), Bundesanstalt fur Gewissenschaften und Rohstoffe (1986), Macdonald *et al.* (1988), and Van Dover ("ALVIN-RISE Cruise Report, 1988; unpublished). Guaymas data is from Grassle (1986). Guaymas *Riftia* have been tentatively identified as an undescribed new species by M.L. Jones (personal communication). Guaymas decapods were identified by A.B. Williams (1988; personal communication). Guaymas clams in the family Vesicomyidae were originally identified as *Calyptogena pacifica*. Analysis of recent collections of vent and seep bivalves indicate that this identification must be revised to *Vesicomya gigas* (R.D. Turner, personal communication).

Across 40 degrees of latitude, there is a remarkable consistency at the familial level in the megafaunal composition of vent communities. Vesicomyid clams and munidopsid squat lobsters, for example, are known from each of the 6 locations listed in Table 4. Riftiid tubeworms are apparently absent only at the southern-most location, while bythograeid crabs do not occur at Guaymas, the northern-most extreme. Mytilid mussels are missing from the two most northern sites.

Even considerable differences in habitat, such as those encountered in the hydrocarbon-rich, sediment-hosted environment of Guaymas Basin vents, do not seem to have significant effects on the familial character of the megafaunal community: Vesicomyids, munidopsids, and riftiids all occur at Guaymas; mytilids are absent, but they do not occur at 21°N, either. On the other hand, species composition of Guaymas Basin megafauna (*Vesicomya gigas*, *Munidopsis alvisca*, *Riftia* sp.) is unique compared to the other locations.

There are sites along the East Pacific Rise where towed camera arrays have captured images of concentrations of megafauna that are unusual in being comprised of only a single, or perhaps two species. Lonsdale (1977 and in prep.) reports isolated, dense populations of anemones on the ridge crest at 3.5°S and 4°N, and Van Dover *et al.* (1987) mention assemblages of ophiuroids and brachyuran crabs at several ridge crest sites near 10°45'N. Entangled masses of enteropneusts and galatheid crabs characterize the fauna of the ridge axis between 11°16 and 11°18'N on the EPR (Van Dover, unpublished). Without more detailed surveys, it is difficult to decide if these unusual faunal assemblages comprise entire vent communities, or whether the camera array has simply skirted the center of a more typical vent field, or if unusual flow regimes have concentrated suspended food particles independent of any hydrothermal activity. In some instances, these sightings are correlated with temperature anomalies and therefore are demonstrably hydrothermal situations. If these observations do indeed include the core of the vent field, these unusual faunal compositions may result from environmental and/or biological circumstances unlike anything we have studied so far. There is also documentation of conventional vent

taxa in relatively unconventional places, viz. vesicomyid clams in the Tamayo Fracture Zone (Gallo et al., 1984 and photographs from ALVIN dive #977). These, too, await explanation.

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Table 1. Galapagos Vent Fields: comparison of megafauna.

KEYS:

D = dominant (biomass)	RG79 = Rose Garden '79
A = abundant	CB1/MB = Clambake 1/Musselbed
M = moderately abundant	SF = Small Fry
R = rare	CB2 = Clambake 2
+ = present	DP = Dandelion Patch
- = absent	OB = Oyster Bed
blank = unknown.	GoE = Garden of Eden

	RG79	CB1/MB	SF	CB2	DP	OB	GoE
WARM-WATER VENT SPECIES							
Riftia pachyptila	D	M	-		-	A	D
Calyptogena magnifica	A	M	-		-	D	dead
Bathymodiolus thermophilus	A	D	-	D	-		R
Neomphalus fretterae	-	A	-				A
?Diplacanthopoma	R	A	A				A
NEAR-FIELD SPECIES							
Anemones*	A	-?	-			M/R	A
Serpulids*	A	A	M	+?	-	A	A
Bathypecten ?vulcani	M						
Turrid Gastropod	R						
PERIPHERAL SPECIES							
Thermopalia taraxaca	M	M	A		D	A/M	A
Saxipendium coronatum	M	A/M	+				
OTHER SPECIES							
Bythograea thermydron	A	A	R				A
Munidopsis subsquamosa	A	A	A				M
Thermarces sp.	Present (R), but vent field unknown.						

Distances between Galapagos Vent Fields
Progressing East to West

Rose Garden to Clambake 1/Musselbed	8000 m
Clambake 1/Musselbed to Small Fry	"near"
Clambake 1 to Clambake 2	1600 m
Clambake 2 to Dandelion Patch	250 m
Dandelion Patch to Oyster Bed	250 m
Oyster Bed to Garden of Eden	650 m
TOTAL	10750 m

*More than one species present.

Table 2. East Pacific Rise Vent Fields, 13°N: comparison of megafauna.

KEYS:
+ = present
- = absent
blank = unknown

PR = Pogoroux
PA = Parigo
PM = Pogomort
PS = Pogosud
AC = Actinoir
PN = Pogonord

	PR	PA	PM	PS	AC	PN
WARM-WATER VENT SPECIES						
Riftia pachyptila	+	+	-	+	+	+
Tevnia jerichonana		+	dead	+		+
Calyptogena magnifica	-	-	dead	-	dead	-
Bathymodiolus thermophilus	-	+	+	+		+
Neomphalus fretterae		+	-			
?Diplacanthopoma		+	-		+	
HIGH-TEMPERATURE CHIMNEY SPECIES						
Alvinella pompejana	+	+	-		+	+
Cyanagraea praedator	+	+	-	-	+	+
NEAR-FIELD SPECIES						
Anemones*		+	-		+	+
Serpulids*	+	+	+	+	+	+
Bathypecten vulcani			+	+		
Turrid gastropod		+	+	+		
Neolepas zevinae		+	+			
Ophiuroid		+		+		
PERIPHERAL SPECIES						
Thermopalia ?taraxaca			+	+		
OTHER SPECIES						
Bythograea thermydron	-	+	-	+	-	+
Munidopsis subsquamosa	+	+	+	+	+	+
Thermarces sp.	+	+	+	+	+	+

Distances between 13°N Vent Fields
Progressing South to North

Pogoroux to Parigo	200 m
Parigo to Pogomort	20-30 m
Pogomort to Pogosud	50 m
Pogosud to Actinoir	300 m
Actinoir to Pogonord	480 m
TOTAL	1080 m

*More than one species present.

Table 3. East Pacific Rise Vent Fields, 21°N: comparison of megafauna.

KEY:
D = dominant (biomass)
A = abundant
M = medium
R = rare
blank = unknown

	Clam Acres	Hanging Gardens
WARM-WATER VENT SPECIES		
Riftia pachyptila	M	D
Oasisia alvinae	R	A
Calyptogena magnifica	D	M
HIGH-TEMPERATURE CHIMNEY SPECIES		
Alvinella pompejana	A	
Munidopsis lentigo	M	
NEAR-FIELD SPECIES		
Serpulids*	R	A
Coiled gastropod	A	
Neolepas zevinae	R	A
PERIPHERAL SPECIES		
Sponges*	-	A
OTHER SPECIES		
Bythograea thermydron	M	M
Munidopsis subsquamosa	A	A
Thermarces sp.	A	

Distance between Clam Acres and Hanging Gardens: 7000 m

*More than one species present.

Table 4. Comparison of vent fauna on ridge segments.

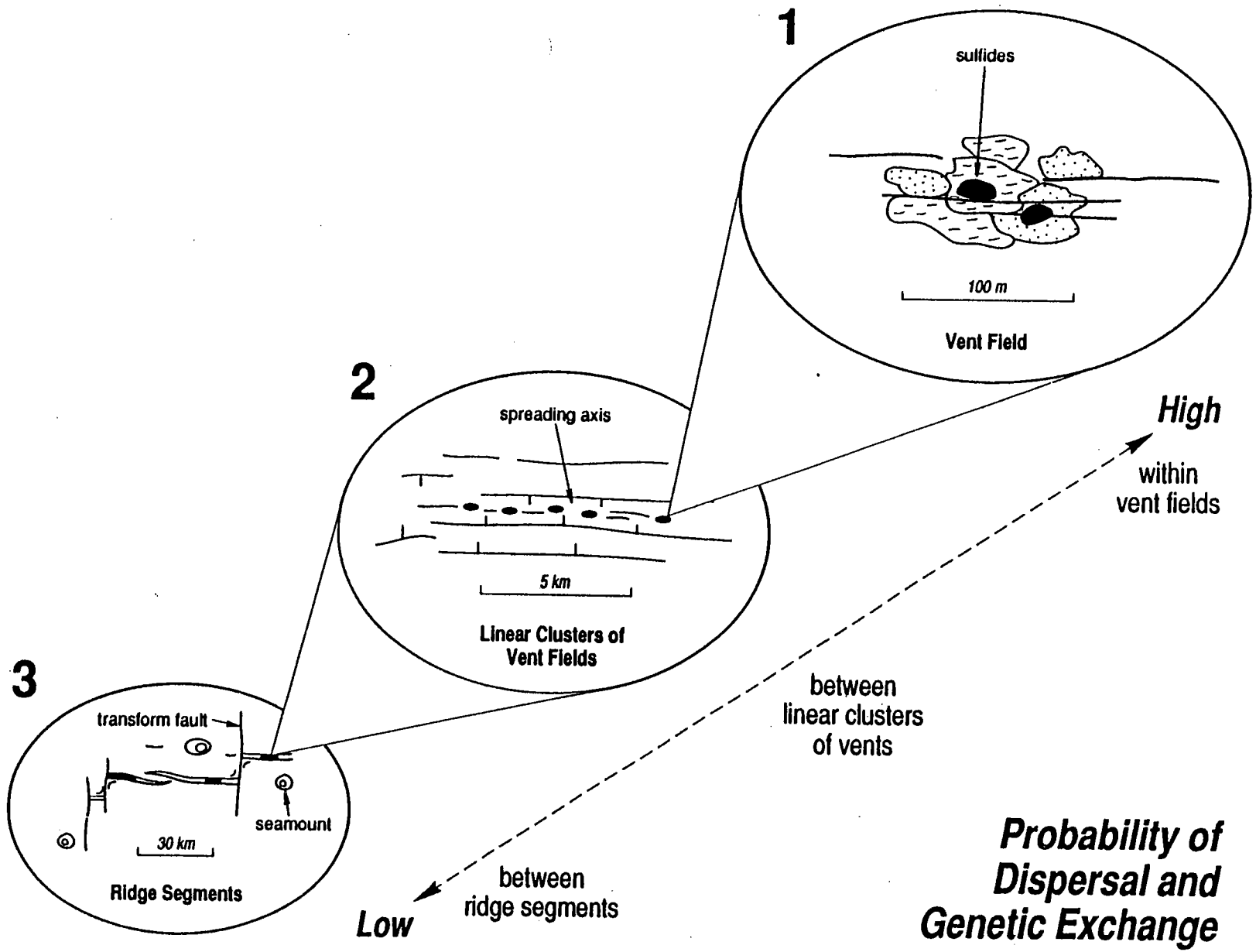
KEY: GY = Guaymas
GA = Galapagos
Numeric designations refer to degrees latitude, EPR

	GY	21N	13N	10-12N	GA	17-20S
VESTIMENTIFERAN TUBE WORMS						
Riftia pachyptila		+	+	+	+	
Riftia (undescribed n. sp.)	+					
Tevnia jericohnana			+	+		+
Oasisia alvinae		+				
BIVALVE MOLLUSKS						
Vesicomya gigas (formerly identified as *Calyptogena pacifica*)	+					
Calyptogena magnifica		+	+*	+	+	
Calyptogena sp.						+
Bathymodiolus thermophilus			+		+	
Bathymodiolus sp.				+		+
Idasola sp.	+					
ANOMURAN AND BRACHYURAN DECAPOD CRUSTACEANS						
Neolithodes diomedeae	+					
Munidopsis subsquamosa		+	+	+	+	
Munidopsis lentigo		+				
Munidopsis alvisca	+					
Munidopsis sp.						+
Bythograea thermydron		+	+	+	+	
Bythograea microps			+			
Bythograea sp.						+
Cyanagraea praedator			+	+		

*Shells only

Figure Legend

Figure 1. A three-tiered hierarchy of 1) individual vent fields, 2) clusters of vent fields on a ridge segment, and 3) ridge segments provides the context in which distributions of vent megafaunal species is discussed. Underlying this spatial hierarchy is a gradient in the probability of dispersal and genetic exchange. At global and regional scales, these processes are most likely to control the faunal composition of vent communities.


1
sulfides
100 m
Vent Field
2
spreading axis
5 km
Linear Clusters of
Vent Fields
3
transform fault
seamount
30 km
Ridge Segments
High
within
vent fields
between
linear clusters
of vents
between
ridge segments
Low
Probability of
Dispersal and
Genetic Exchange

Chapter 3

Hydrothermal vent fauna of Escanaba Trough (Gorda Ridge)

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Biota of the NESCA site, Escanaba Trough

Hydrothermal vent communities on Gorda Ridge were discovered and documented during 1988 field programs using the deep-diving submersibles *Alvin* and *Sea Cliff*. Venting was associated with large sulfide mounds that comprise the NESCA site in the Escanaba Trough of Gorda Ridge (41°00'N; 127°29'W; 3200-3250 m). Details of the geological setting are presented elsewhere in this symposium volume.

Within the study area, we identified 5 principle habitats:

1) Active hydrothermal venting associated with massive sulfide deposits (temperatures ranged from a few degrees above ambient to a maximum of 220°C);
2) A low-temperature site associated with soft sediment deposits at the base of sulfide mounds;
3) 'Inactive' sulfide mounds;
4) Non-vent soft sediment;
5) Non-vent basalt lava.

The fauna of active hydrothermal vents associated with sulfide deposits was lush, dominated by slender vestimentiferan tubeworms in the family Ridgeiidae. Tubeworm tubes were heavily fouled by paralvinellid and ampharetid polychaetes. We noted local differences in the composition of macrofaunal species associated with different tubeworm clumps: At some sites, the distal ends of the tubeworm tubes were ornamented with one or two white-bodied anemones with pink-fringed tentacles. Some clumps of tubeworms were colonized by abundant lepetodrilid limpets and small, coiled gastropods; in other clumps, these mollusks were rare or absent. At the vent site designated 6X,

three arthropod taxa were abundant within the tubeworm clumps: Pycnogonids of all size classes, including gravid females and juveniles were found attached to tubeworm tubes; red copepods were found in mucous and washings of tubeworm clumps; large tanaid crustaceans encased in thin mud tubes also fouled tubeworm tubes. The tanaids belong to the family Neotanaidae and represent, to our knowledge, the first recorded collection of a vent-associated tanaid species. Tanaids of all size classes were collected, from small ~3 mm juveniles to large ~1.5 cm adult males. Polynoid polychaetes were common but not abundant in all clumps of tubeworms. Copious mucous secretions by paralvinellid polychaetes at the base of tubeworm clumps appeared to form a matrix within which filamentous bacteria thrived. Populations of paralvinellid worms were also observed in tubes within sulfides adjacent to active vents where tubeworms were absent. Disk-like sponges (1-2 cm diameter) were abundant on sulfides surrounding areas of hydrothermal venting. A sample of fossilized worm tubes embedded in iron oxides near a 110°C vent was colonized by dense populations of small ampharetid polychaetes and aplacophoran mollusks. Small vestimentiferan tubeworms, anemones, limpets, pycnogonids, and folliculinid protozoans also colonized this substrate. Galatheid crabs reached their greatest densities in the vicinity of active venting on sulfide mounds.

One site of low-temperature venting in soft sediment was observed at the base of a sulfide mound. The area (< 10 m^2) was colonized by elongate vesicomyid clams identified as *Calyptogena phaseoliformis* by R.D. Turner. Live clams were oriented half-buried, with their long axes at about 45° angles with respect tc the sediment surface. The exposed anterior ends of live clams were often colonized by anemones. A number

of empty valves lying on the surface of the sediment were noted, but no obvious explanation for mortality was observed. Qualitative samples of the sediment at this site contained large populations of undescribed species of orbiniid, ampharetid, and spionid polychaetes.

'Inactive' sulfide mounds were often colonized by remarkably dense concentrations of suspension-feeding deep-sea fauna. This fauna included aggregations of large, solitary tunicates plus brisingid seastars, crinoids, sponges, anemones, and brachiopods. While these organisms are common elements of the local non-vent fauna (Cary, this volume), their unusual abundance on sulfide mounds suggests that there may be some low-level venting of hydrothermal fluids at these 'inactive' sites that supports chemosynthetic production within the overlying water column, or that the acid-labile sulfides are mobilized by microbial activity to support primary production. Alternatively, the topographic relief of the sulfide mounds may modify the local flow regime, concentrating suspended particulates on which the biota feed.

Non-vent soft sediments were heavily bioturbated. Echinoderms, including asteroids, ophiuroids, holothurians, and urchins were conspicuous elements of the megafauna, as were xenophyophores and anemones. Occasional pennatulaceans and galatheid squat lobsters were also observed. The infauna of the sediments was dominated by several small species of polychaetes and an isopod.

Fauna of non-vent basalt substrates included sponges, brachiopods, and sabellid polychaetes.

Faunal Affinities

Specific identifications of most of the Gorda Ridge biota remain to be confirmed by taxonomic specialists. Nevertheless, we can consider the general nature of the fauna and relate it to the fauna of vent communities described from other oceanic spreading centers. Not unexpectedly, the fauna of NESCA hydrothermal vents most closely resembles that of Juan de Fuca and Explorer Ridge vent communities. The Northeast Pacific assemblage of Tunnicliffe (1988) can be extended to embrace the Gorda Ridge fauna. Ridgeiid vestimentiferans, lepetodrilid limpets, paralvinellid, polynoid and ampharetid polychaetes, pycnogonid arthropods and the small coiled gastropod are important components of this assemblage. Common and abundant faunal types present at vents on Juan de Fuca and Explorer Ridges but so far characterized as absent or rare at the NESCA site on Gorda Ridge include maldanid polychaetes and ostracods. The majid crab, *Macrooregonia macrochira*, while not strictly a vent-associated species, was not observed at NESCA, though it is common at Juan de Fuca and Explorer vents. Tanaid crustaceans occur at the NESCA site but have not been noted at Juan de Fuca or Explorer Ridge vents.

The fauna of the low-temperature soft-sediment site at NESCA, dominated by *Calyptogena phaseoliformis*, is not part of Tunnicliffe's Northeast Pacific assemblage. The same species of clam is known from cold water seeps in soft-sediment off Japan (Metivier et al. 1986; Juniper & Sibuet 1987) and was recently identified from sites in Monterey Canyon off California (R.D. Turner, pers. comm.). Soft-sediment vent fauna is best known from Guaymas Basin (Gulf of California) where the clam *Vesicomya gigas* (previously identified as

California) where the clam *Vesicomya gigas* (previously identified as *Calyptogena pacifica* and reclassified by R.D. Turner on the basis of additional material) is abundant (Grassle et al. 1985). Guaymas sediments are infused with petroleum hydrocarbons formed from high-temperature cracking of recent organic material (Simoneit and Lonsdale 1982); an odor of petroleum was detected in Gorda vent sediment, but it was neither as strong nor as pervasive as that of Guaymas material. The single, qualitative sample of Gorda vent sediment shares no common infaunal taxa with the samples of vent sediment from Guaymas Basin and the extensive bacterial mats associated with the sediment surface at Guaymas are absent at the Gorda soft-sediment vent site.

Acknowledgements

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Chapter 4

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Recruitment of marine invertebrates to hard substrates at deep-sea hydrothermal vents on the East Pacific Rise and Galapagos spreading center

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Abstract—Recruitment panels were placed at and near hydrothermal vent communities at three sites on the Galapagos spreading center and one site on the East Pacific Rise at 21°N. Deployment periods ranged from 26 days (Clam Acres, 21°N) to 260–320 days (Rose Garden, Garden of Eden, Mussel Bed, GSC) to 1216 days (Clam Acres). Recruitment of gastropod post-larvae and juveniles was observed on arrays deployed at Clam Acres for 26 days. Regardless of length of deployment, populations of polychaetes, mollusks, and barnacles colonizing the panels were predominantly post-larval, juvenile, or sub-adult stages. We suggest that some combination of competition, migration, and predation maintains these populations in immature stages. Size distributions of individuals within a taxon on panels deployed for 1216 days are broad, suggesting intermittent or continuous recruitment in many of the vent-associated species rather than a single episodic recruitment event. Folliculinid and foraminiferan protozoans were the most abundant eucaryotic organisms colonizing long-term deployments at Clam Acres. On the Galapagos spreading center, level of recruitment differed among the vent sites, with Rose Garden > Garden of Eden ≫ Mussel Bed. Recruitment of vent-associated species was greater on panels placed within vent communities compared to panels placed adjacent to these communities. This observation is consistent with the maintenance of vent communities in discrete regions of hydrothermal flux.

INTRODUCTION

HYDROTHERMAL vent communities on the East Pacific Rise at 21°N and on the Galapagos spreading center (GSC) consist of dense populations of megafaunal and macrofaunal invertebrates centered around venting hot (350°C) and/or relatively warm (5–17°C) water. Vent communities are characterized by three important physical attributes: they are spatially discrete, they are geographically isolated, and they are ephemeral [see GRASSLE (1986) for a review of vent characteristics]. Given these attributes, how are the dense populations of invertebrates maintained at vent sites?

One aspect of this problem has been studied, namely, the nature of the dispersive stages of vent fauna. Many of the conspicuous vent species at 21°N and on the GSC are relatively sessile and appear to be endemic to vents, not occurring on adjacent hard-substrate terrain (FRANCE and VAN DOVER, unpublished data). Such species undoubtedly rely on larval stages for dispersal and maintenance of populations. LUTZ *et al.* (1984),

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summarizing the work of various investigators, note that vent fauna exhibit an array of larval types, with some species thought to undergo highly dispersive planktotrophic development, but with most undergoing less dispersive, non-planktotrophic development. Predominance of non-planktotrophy has been attributed to phylogenetic constraints on vent taxa rather than to the nature of the vent habitat itself (BERG, 1985; TURNER *et al.*, 1985; VAN DOVER *et al.*, 1985). BERG and VAN DOVER (1987) document the presence of dispersive larval and juvenile stages of vent organisms in the water column overlying established vent communities at 21°N and on the GSC. Larval and post-larval stages of vent invertebrates also have been collected in plankton tows above the Guaymas Basin vent area in the Gulf of California (BERG and VAN DOVER, 1987; WIEBE *et al.*, 1988).

Production and dispersal of offspring are only part of the sequence of events that ensures maintenance of invertebrate populations. Another important aspect involves settlement and recruitment processes. Initial impressions of megafaunal populations at vents, notably of the clam, *Calyptogena magnifica* Boss and Turner, suggested that populations at a given vent are derived from a single, episodic recruitment event (CORLISS *et al.*, 1979). This conclusion was based on what appeared to be remarkably uniform sizes of individuals. Subsequent detailed observations indicate that the situation is more complex (HESSLER and SMITHEY, 1983). Competition (HESSLER *et al.*, 1985) and predation (RHOADS *et al.*, 1982) are thought to play significant roles in determining population dynamics and the nature of the vent community in terms of its species composition and relative abundances of species. These intra- and interspecific interactions among adults may obscure patterns of recruitment. Superimposed on the complexities of biological interactions are the subtle and the potentially catastrophic variations in geochemistry and water flow at vents that influence settlement, recruitment, and survival of vent organisms.

In this paper, we examine species composition and abundance of recruits to slate panel arrays deployed by the submersible *Alvin* at the near vent communities on the East Pacific Rise at 21°N and on the GSC. Because of difficulties in obtaining true replicates and in eliminating interaction effects due to variations in local flow regimes, we restrict our discussion to qualitative comparisons of species composition and abundance among treatments.

METHODS

Panel arrays were designed to yield information concerning colonization of new, clean, hard substrates similar in texture to seafloor basalt near vent sites. Arrays consisted of four slate panels (6 mm × 15 cm × 15 cm) oriented horizontally on Eccofloat syntactic foam blocks of slightly larger dimensions and separated by 6.4 cm lengths of polyvinylchloride tubing (Fig. 1). Slate (from Plymouth Quarries, MA) was chosen as the substrate because it is inert and easily cut into thin slabs. Each array was tethered by polypropylene line to a 4.5 kg lead weight at the anchoring end, with a monkey-fist knot or syntactic foam block at the opposite end. This arrangement of slates ensured that surfaces floated more or less horizontally in uneven terrain. Prior to deployment, slate panels were washed, rinsed with deionized water, and sterilized in an autoclave. Arrays were assembled shortly before deployment and were carried to vent sites by the submersible *Alvin*. All manipulations by *Alvin* were carried out by grasping the array at

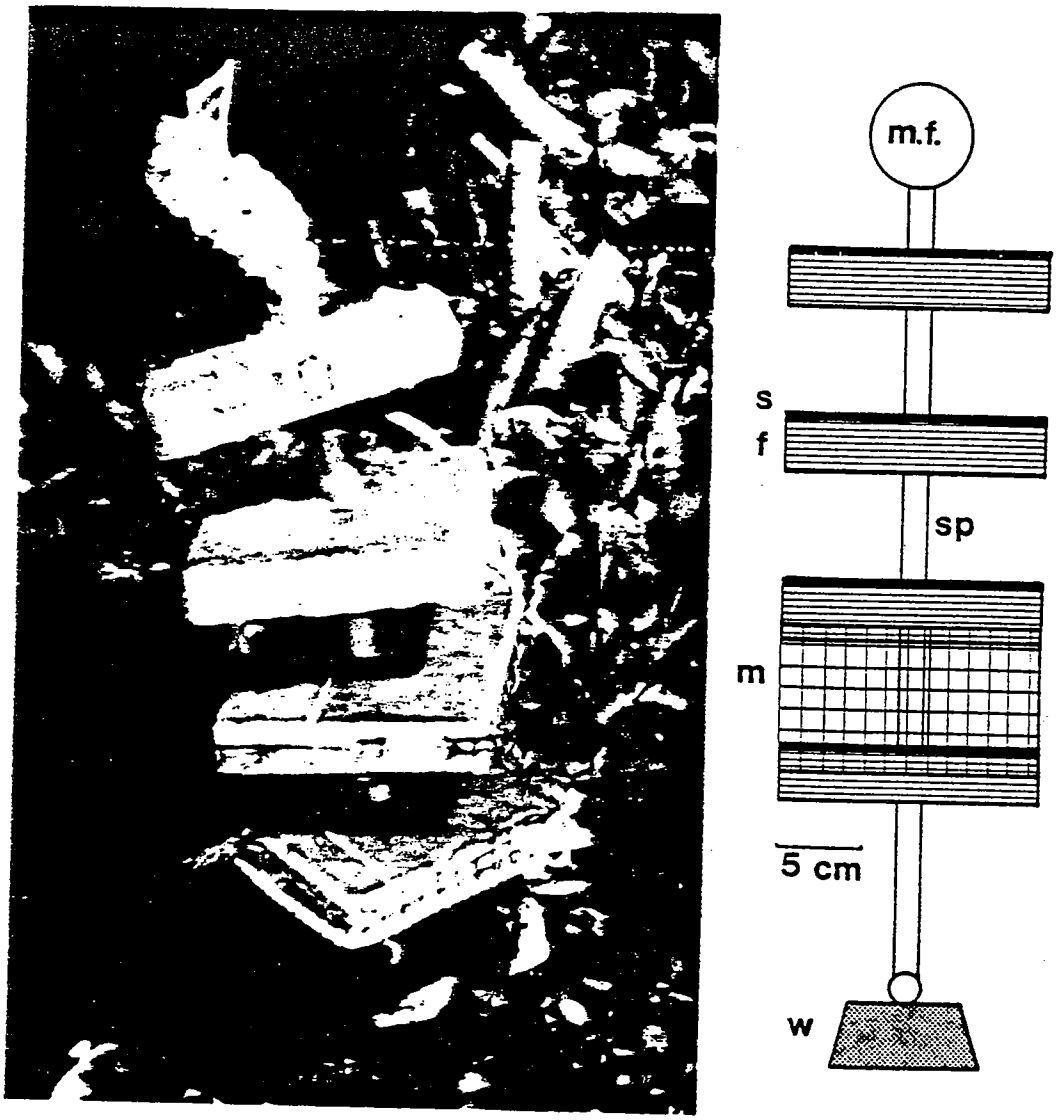

Fig. 1. Array 10 at Rose Garden immediately before its recovery after 261 days *in situ*. Photograph by R. D. Turner. Schematic diagram of a recruitment array: m.f., monkey-fist (replaced by a syntactic foam block on Clam Acres arrays); s, slate slab; f, syntactic foam; sp, space; m, mesh; w, lead weight. Mesh was present on only two of the arrays deployed.

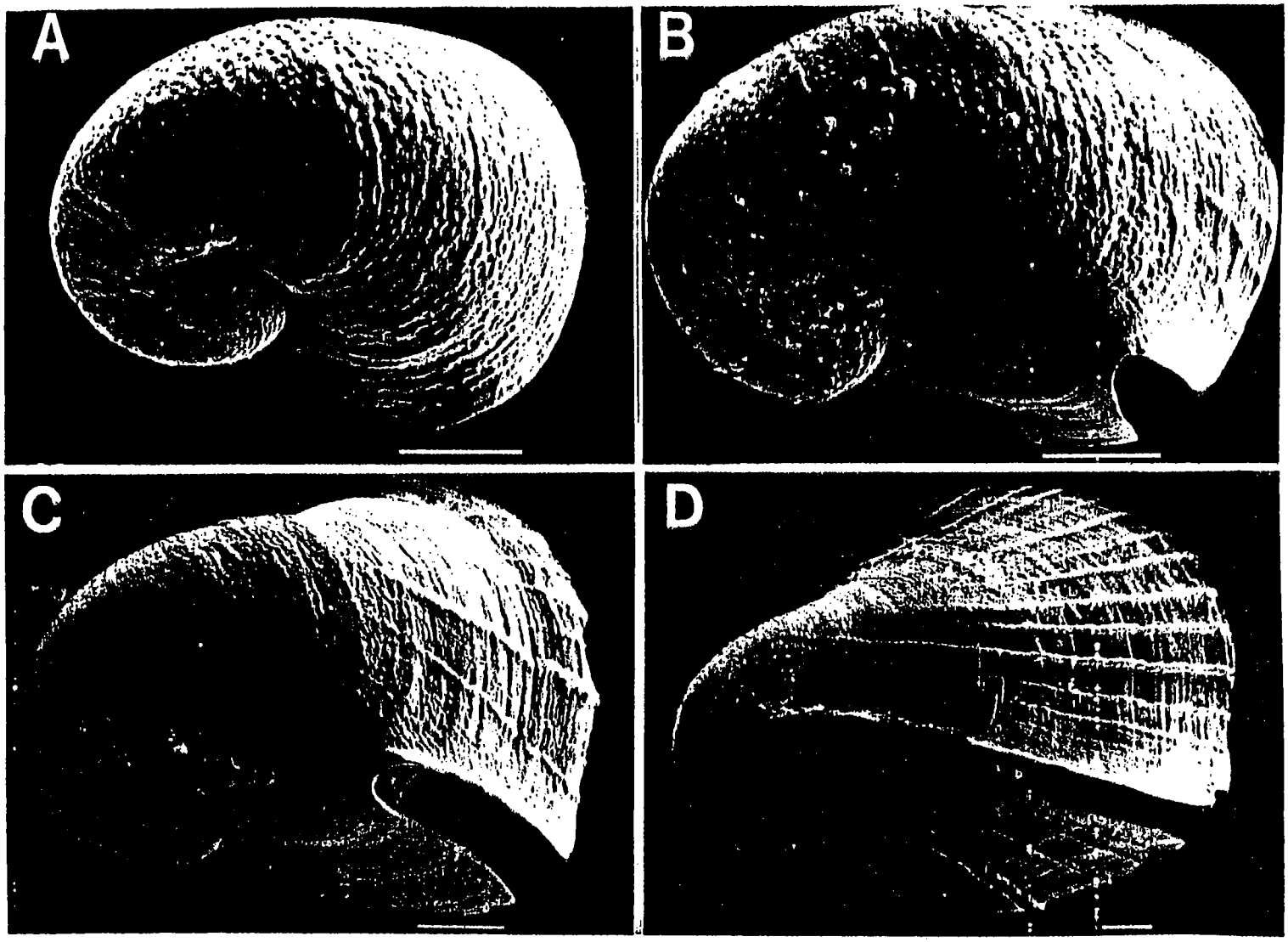

Fig. 2. Ontogenetic sequence (A–D) of fissurellid limpets collected on a single long-term deployment of an array at Clam Acres. Scale bars = 100 μm.

the knot or foam block. Retrieval was accomplished by placing an entire array into a specially designed polyethylene box with a closing lid. In this way, panels were not subject to washing during the ascent of the submersible.

Upon reaching the surface, each array was preserved in its collecting box, or was removed, disassembled and preserved as separate components. Either 7% buffered formaldehyde or 5% buffered glutaraldehyde was used as a preservative, with storage after sorting in buffered 70 or 80% EtOH. Panels and washings sieved through a 0.333 μm mesh sieve were examined under a dissecting microscope. All animals were identified and counted. Identifications and descriptions of new species were made by taxonomic specialists (e.g. MACIOLEK, 1981; BLAKE, 1985; MCLEAN, 1985, 1988, in press; SMALL and GROSS, 1985). Coiled gastropods are described by WAREN and BOUCHET (in press). Juvenile specimens of bivalves from Clam Acres were assumed to be *Calyptogena magnifica* since no other species of bivalve is known from this site. At Galapagos vents, clam juveniles could be readily distinguished from mussel and scallop juveniles. Galapagos polychaete and limpet fauna were not identified to species, a consequence of the small size of specimens and their poor condition after several years of storage. Similar difficulties were encounted with invertebrates collected on arrays deployed for brief periods at Clam Acres. Elsewhere, when species designations are not provided, the animals usually belong to undescribed new species.

Arrays were placed at three separate vent sites on the GSC in 1979: Rose Garden, Mussel Bed, Garden of Eden. Figure 1 includes a photograph of one of these arrays *in situ*, just prior to recovery. The schedule of deployments and retrievals is given in Table 1. At each site, at least one array was placed within the region of hydrothermal activity and the biological community (= 'vent') and another was placed several meters away from the community in an area of relatively barren lava (= 'non-vent'). The vent sites and their communities have been described and illustrated elsewhere (GRASSLE *et al.*, 1979; GRASSLE, 1986; CRANE and BALLARD, 1980; HESSLER and SMITHEY, 1983). Arrays were recovered after approximately 9 months (Rose Garden) or 11 months (Mussel Bed, Garden of Eden).

Table 1. *Deployment and retrieval of recruitment panel arrays at deep-sea hydrothermal vents*

Array no.	Location	Site	Deployment Dive: date	Retrieval Dive: date	Days elapsed
Galapagos Rift, 0°48'N, 86°09'W, 2450 m					
1	Mussel Bed	Vent	880: 21/01/79	987: 04/12/79	317
4	Mussel Bed	Vent	880: 21/01/79	989: 06/12/79	319
8	Mussel Bed	Non-vent	882: 23/01/79	987: 04/12/79	315
9	Mussel Bed	Non-vent	882: 23/01/79	991: 08/12/79	319
2	Garden of Eden	Vent	883: 24/01/79	993: 10/12/79	320
5	Garden of Eden	Vent	883: 23/01/79	993: 10/12/79	320
6	Garden of Eden	Non-vent	884: 25/01/79	993: 10/12/79	319
10	Rose Garden	Vent	905: 15/03/79	984: 01/12/79	261
7	Rose Garden	Non-vent	905: 15/03/79	983: 30/11/79	260
East Pacific Rise, 20°50'N, 109°06'W, 2600 m					
1	Clam Acres	Vent	1213: 19/04/82	1229: 14/05/82	26
2	Clam Acres	Vent	1213: 19/04/82	1229: 14/05/82	26
9	Clam Acres	Vent	1222: 06/05/82	1634: 03/09/85	1216
10	Clam Acres	Vent	1222: 06/05/82	1634: 03/09/85	1216
3	Clam Acres	Non-vent	1213: 19/04/82	1227: 11/05/82	22
4	Clam Acres	Non-vent	1213: 19/04/82	1227: 11/05/82	22

Arrays also were deployed at and near ('vent' and 'non-vent') Clam Acres on the East Pacific Rise at 21°N in 1982. The biology of this site is described in HESSLER *et al.* (1985). Some of these arrays were recovered after approximately 3 weeks while others were left in place for 3.3 years. See Table 1 for details of the deployment schedule.

Observations of biological activity around arrays and of arrays themselves were made by scientists in *Alvin* during diving operations. Additional photographs of arrays *in situ* have been published in HESSLER *et al.* (1985; Fig. 9) and JANNASCH (1985; Fig. 3).

Our initial intent was to analyse each panel of each array independently to establish whether height above the bottom was an important determinant in recruitment processes at vents. Upon recovery of arrays, it was obvious that very few species actually adhered to the panels. The majority of animals associated with the arrays were to be found in sieved (0.333 μm mesh) washings from the bottom of each retrieval box. For this reason, after initial assessment of attached organisms on each panel, data from all panels of an array and in washings from that array were pooled as a single treatment.

The bottom panel of arrays 1 and 9, deployed at Mussel Bed, were enclosed by 1 cm^2 mesh of black plastic. On retrieval of these arrays, it was discovered that the mesh had disintegrated (Fig. 1). We have chosen to treat these arrays as if the mesh had not been present since (i) we could discern no difference between the fauna colonizing the bottom panel and that of the other three panels, (ii) most of the animals associated with each array were to be found in the bottom of the retrieval boxes, and (iii) we have no way of knowing when the mesh disintegrated.

RESULTS

Galapagos spreading center

Recruitment arrays left in place at and near Galapagos hydrothermal vent sites for 260–321 days were not covered by megafaunal invertebrates; in fact, *in situ* observations of the arrays from *Alvin* gave the initial impression that no recruitment had taken place at any site. Subsequent examination of washings from the nine arrays yielded nearly 10,000 macrofaunal individuals belonging to 40 invertebrate taxa, including juveniles of two dominant megafaunal bivalves (*Bathymodiolus thermophilus* Kenk and Wilson and *Calyptogena magnifica* Boss and Turner). Details of composition and abundance of invertebrates recovered from each array is given in Table 2, with summary data provided in Table 3.

Within the polychaetous and molluscan taxa, consistent patterns of composition and abundance can be discerned. All of the taxa recovered in these two groups were also recovered from washings of tubeworms, mussels, and clams from Galapagos vents brought to the surface by *Alvin* (H. SANDERS and I. WILLIAMS, personal communication); they are clearly vent-associated fauna, although their endemicity is brought into question by their recovery as juveniles on arrays placed beyond the presumed limits of the vent communities. Most of the individuals were juveniles or sub-adults. Diversity of polychaetes and mollusks (defined simply as the number of taxa) on arrays at vent sites was always equal to or greater than diversity of polychaetes and mollusks on arrays located adjacent to vent sites. Abundances of polychaetes and mollusks on arrays within vent sites were always at least twice (typically more) their abundances on arrays adjacent to the corresponding vent sites.

In between-vent comparisons, Mussel Bed is distinguished by having the lowest

Table 2. Number of animals collected from recruitment arrays deployed for 260–321 days at Galapagos spreading center vent sites. V, vent; N-V, non-vent; +, presence of colonial species

	Mussel Bed				Rose Garden		Garden of Eden		
Array no.	1	4	8	9	10	7	2	5	6
Locality	V	V	N-V	N-V	V	N-V	V	V	N-V
Protozoa									
Foraminifera	6	5	1		2	6	2		
Coelenterata									
Hydrozoa			+	+	+				+
Anthozoa		5	1		31	7	6	5	2
Platyhelminthes									
Turbellaria					7	6			
Nemertea		1		1	30	9	2	6	
Nematoda	1								
Annelida									
Polychaeta									
Euphrosinidae	2	1		1	284	148	2	12	
Polynoidae		2			45	11	1	8	
Phyllodocidae					4			6	
Hesionidae		9	1	2	301	84	12	27	2
Nereidae		1			19	9	1	13	
Dorvilleidae		3		2	2438	113	52	512	2
Maldanidae					1	1	1	1	
Ampharetidae	2	1			31	5	20	26	
Terebellidae							2		
Serpulidae	7								
Sabellidae			1		167	1		1	
Spionidae	3	21		1	353	206	95	187	3
Hirudinea								1	
Mollusca									
Gastropoda									
Limpets									
Fissurellidae	11	14			290		2	21	5
Group C symmetrical		1			12	58	3	44	1
Lepetodrilaceans	3	4			47	2	1	7	5
Decalcified					16		1	5	3
Coiled									
Gastropod A					2				3
Gastropod B	1							7	
Gastropod C					175		2	5	
Gastropod D					8				
Nudibranch				1					
Pelecypoda									
Bathymodiolus thermophilus	5	15	7	1	13	15	28	42	7
Calyptogena magnifica		2	2	1	15		1	1	
?Bathypecten vulcani	2						1		
Aplacophora									
Simrothiella sp.					2	2	2	1	
Arthropoda									
Crustacea									
Ostracoda				1		157			
Copepoda	3	3	2	172	14	3206	2	11	1
Leptostraca								1	
Tanaidacea	1		1						
Isopoda	3	20	1	3			1		3
Amphipoda		2	1	10	9			1	6
Decapoda									
Galatheidae				1					
Chelicerata									
Pycnogonida				1					

See Table 3 for totals.

Table 3. Summary of invertebrate composition and abundance on recruitment arrays deployed for 260–321 days (see Table 1) on the Galapagos spreading center. V, vent; N-V, non-vent

	Mussel Bed				Rose Garden		Garden of Eden		
Array no.	1	4	8	9	10	7	2	5	6
Location	V	V	N-V	N-V	V	N-V	V	V	N-V
Total number of taxa	14	18	11	15	27	19	23	25	14
Total number of individuals	50	110	18	198	4316	4046	240	950	43
Total number of polychaete taxa	4	7	2	4	16	9	9	10	3
Total number of mollusk taxa	5	5	2	3	10	4	9	9	6
Total number of polychaetes	14	38	2	6	3643	578	186	793	7
Total number of mollusks	22	36	9	3	580	77	41	133	24

diversity and abundance of polychaetes and mollusks. Diversities of polychaetes and mollusks on Rose Garden and Garden of Eden arrays were similar, but abundances were greater on the Rose Garden arrays. This is despite the fact that Rose Garden arrays were in place for the shortest period of time (260 vs ~320 days). Four polychaetous taxa (Spionidae, Dorvilleidae, Hesionidae, Euphrosinidae) and four molluscan taxa (Fissurellidae, *Bathymodiolus*, *Calyptogena*, Group-C symmetrical limpet) were dominant in terms of abundance, comprising ≥10% of total polychaetes + mollusks on a single array (Table 4).

Patterns in composition or abundance among the remaining taxa are difficult to pick out. There is an overall greater abundance of Crustacea on non-vent arrays than on vent arrays: a large number of zooplankters (copepods and ostracods) were observed *in situ* and recovered from the non-vent Rose Garden array; relatively large numbers of copepods also were recovered from one of the non-vent Mussel Bed arrays. Distributions

Table 4. Ranked dominant taxa (polychaetes and mollusks): taxa comprising ≥ 10% of the total number of polychaete and mollusk individuals at a given site on the Galapagos spreading center; abundances from duplicate recruitment arrays were combined

Mussel Bed			
Vent (arrays 1 and 4)		Non-vent (arrays 8 and 9)	
Fissurellidae	(23%)	*Bathymodiolus*	(42%)
Spionidae	(21%)	*Calyptogena*	(16%)
Bathymodiolus	(18%)	Hesionidae	(16%)
Rose Garden			
Vent (array 10)		Non-vent (array 7)	
Dorvilleidae	(52%)	Spionidae	(31%)
Spionidae	(11%)	Euphrosinidae	(23%)
		Dorvilleidae	(17%)
Garden of Eden			
Vent (arrays 2 and 5)		Non-vent (array 6)	
Dorvilleidae	(49%)	*Bathymodiolus*	(24%)
Spionidae	(24%)	Fissurellidae	(18%)
		Group C Symmetrical	(18%)
		Limpet, decal.	(11%)
		Spionidae	(11%)

of other crustaceans show no consistent patterns. Among the lower invertebrate fauna (Protozoa, Coelenterata, Platyhelminthes, Nemertea, Nematoda) there is a tendency toward greater abundances on arrays within vent sites and greatest abundances at Rose Garden, but the numbers are too small to be conclusive.

East Pacific Rise, 21°N

A total of 202 individuals, belonging to 13 taxa, were recovered from four arrays deployed for short periods (23–26 days) at the Clam Acres hydrothermal vent site (Table 5; summary statistics are provided in Table 7). Diversities and abundances of invertebrates were greater for two arrays placed within the clam field than for the two arrays outside the vent field. Relatively large numbers (≥10 individuals on at least one of each pair of arrays) of post-larval and juvenile gastropods were recovered on the vent arrays; copepods, amphipods, and nematodes ranked next in abundance. Copepods were the only faunal component associated with the non-vent arrays in densities of >2 individuals. No polychaetes were recovered from the vent arrays; a single polychaete fragment was recovered from a non-vent array.

Long-term deployments of two recruitment arrays, placed within 2 m of each other in the clam field at Clam Acres for 1216 days (3 years and 4 months), were covered with filamentous bacteria. Species compositions and abundances of invertebrates recovered from these arrays are given in Table 6 (see Table 7 for summary statistics). Two new foraminiferan species were collected. Professor P. BRONNIMANN (Switzerland) is preparing taxonomic descriptions of these protozoans. Copepods, one of the most abundant taxa, were recovered in nearly equal numbers from each array. Other abundant taxa

Table 5. Number of animals collected from recruitment arrays deployed for 23–26 days at Clam Acres vent site on the East Pacific Rise at 21°N. V, vent; N-V, non-vent; +, presence of colonial organism

	Array 1 V	Array 2 V	Array 3 N-V	Array 4 N-V
Protozoa				
Folliculinids		2		
Foraminifera	2			
Coelenterata				
Hydrozoa				+
Medusa	1	1		
Platyhelminthes		1	1	
Nematoda	3	12	1	
Annelida				
Polychaeta				1
Mollusca				
Gastropoda				
Limpets	52	5		
Post-larvae	10	2	1	1
Pelecypoda				
Post-larvae			1	1
Arthropoda				
Crustacea				
Ostracoda				2
Copepoda	29	28	12	18
Amphipoda	19	2	1	1

See Table 7 for totals.

Table 6. Number of animals collected from recruitment arrays deployed for 1216 days at Clam Acres vent site on the East Pacific Rise at 21°N

	Array 9	Array 10
Protozoa		
Folliculinids	Present	>1000
Foraminifera sp. 1	494	49
Foraminifera sp. 2	31	0
Nematoda	135	0
Nemertea	+	0
Polychaeta		
Polynoidae	4	8
Phyllodocidae		
Protomystides papillosa	2	2
Hesionidae		
Hesiolyra bergi	1	0
Hesiospina vestimentifera	6	3
Unidentified juveniles	10	12
Nereidae		
Nereis sandersi	18	13
Dorvilleidae		
Ophryotrocha akessoni	104	299
Glyceridae juveniles	3	2
Flabelligeridae	7	9
Maldanidae		
Nicomache arwidssoni	8	12
Ampharetidae		
Amphisamytha galapagensis	178	253
Alvinella pompejana	1	0
Serpulidae	13	17
Mollusca		
Gastropoda		
Limpets		
Transparent symmetrical	3	8
Lepetodrilus sp. juveniles	31	76
Lepetodrilus cristatus	20	63
Lepetodrilus elevatus	6	2
Lepetodrilus ovalis	19	16
Fissurellid	38	66
Coiled Gastropods		
Melanodrymia aurantiaca	0	1
Trochid (= Figs 92–95 in WAREN and BOUCHET, in press)	3	7
Gastropod species 1 (= ?Figs 68–70 in WAREN and BOUCHET, in press)	27	
Gastropod species 2 (= Figs 6–10 in WAREN and BOUCHET, in press)	2	0
Gastropod species 3 (= Figs 28, 29 in WAREN and BOUCHET, in press)	1	0
Juveniles	131	117
Unidentified (decalcified)	3	4
Pelecypoda		
Calyptogena magnifica	21	20
Aplacophora		
Simrothiella sp.	2	6
Crustacea		
Copepoda	444	450
Cirripedia		
Neolepas zevinae	16	17
Amphipoda		
Lyssianassidae	21	4
Ostracoda	3	0
Larvacea	2	0

See Table 7 for totals.

Table 7. Summary of invertebrate composition and abundance on recruitment arrays deployed at Clam Acres on the East Pacific Rise at 21°N. V, vent; N-V, non-vent

Deployment period	Short-term (23–26 days)				Long-term (1216 days)	
Array no.	1	2	3	4	9	10
Location	V	V	N-V	N-V	V	V
Total number of taxa	6	7	6	7	34	24
Total number of individuals	106	52	18	26	1807	1536*
Total number of polychaete taxa	0	0	0	1	12	10
Total number of mollusk taxa	1	1	2	2	12	10
Total number of polychaetes	0	0	0	1	355	630
Total number of mollusks	62	7	2	2	307	386

* Excluding folliculinids.

were not distributed as evenly: folliculinids, described by SMALL and GROSS (1985), densely covered one panel of array 10; polychaetes and gastropods also were most abundant on array 10 (Table 7). Foraminifera sp. 1, amphipods, and nematodes were recovered in greater numbers from array 9. Dorvilleids (*Ophryotrocha akessoni* Blake) and ampharetids (*Amphisamytha galapagensis* Zottoli) were dominant on both arrays, comprising 24 and 26%, respectively, of the total number of polychaetes and mollusks on the two arrays. Juvenile limpets and other gastropods made up another 15% of the total number of mollusks and polychaetes; the remaining taxa within these two groups each comprised less than 10% of the total number of mollusks and polychaetes. As with Galapagos samples, species recovered from Clam Acres recruitment arrays also have been recovered from washings of adult clams and tubeworms collected in the area by *Alvin* (H. SANDERS and I. WILLIAMS, personal communication). Post-larval clams (*Calyptogena magnifica*; width: $\bar{x} = 320 \pm 30$ μm, $n = 28$), adults of which are by far the most conspicuous megafaunal component of the Clam Acres site, were recorded in moderate numbers from each array.

With few exceptions, polychaetes, mollusks and barnacles recovered from long-term deployments of arrays within the clam field were post-larval, juvenile, or sub-adult stages. Within a given species, ontogenetic sequences (based primarily in size, secondarily on morphology) were recovered from individual arrays, indicating that more than a single age class was represented. This is illustrated for the fissurellid limpet in Fig. 2. Size–frequency histograms for four limpet species and one coiled gastropod further document these observations (Fig. 3). Patterns of size distribution for each species are distinct: in an unidentified species of *Lepetodrilus*, only the smallest size classes prevailed; in *Lepetodrilus cristatus* McLean and fissurellid limpets, smallest size classes were predominant, but larger specimens approaching and including adult size were collected; coiled gastropod sp. 1 (resembling Figs 68–70, 88 in WAREN and BOUCHET, in press) is characterized by a tight distribution of size classes around a mean shell height of 2.0–2.5 mm; finally, in the limpet *Lepetodrilus ovalis* McLean, there are clusters of size classes distributed along the entire range of potential sizes, from juvenile to adult. Mean lengths of nine species of polychaetes recovered from arrays 9 and 10 (Table 8) are a fraction of reported sizes for the corresponding adults. The coefficients of variation were large, ranging from 32 to 88% of the mean lengths.

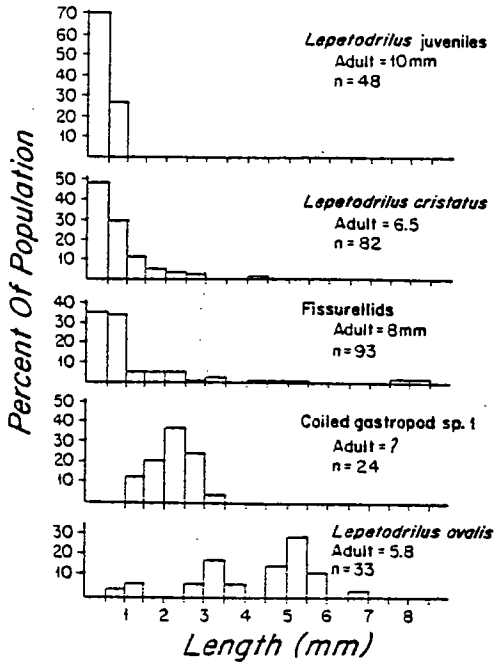

Fig. 3. Size–frequency histograms for four limpet species and one coiled gastropod collected on arrays 9 and 10 at Clam Acres. Adult sizes of Lepetodrilacea were provided by MCLEAN (in press).

Table 8. Lengths of polychaetes recovered from long-term deployments (1216 days) of recruitment arrays at Clam Acres, 21°N. S.D. = standard deviation. C.V. = coefficient of variation

Taxon	$\bar{x}$ (mm)	S.D.	C.V.(%)	n	Maximum size (mm) reported in literature
Polynoidae	1.66	0.70	42	7	12–36*
Protomystides papillosa	0.90	0.43	48	4	>13†
Hesionidae	0.58	0.30	52	19	17–34†‡
Nereis sandersi	0.80	0.62	77	27	95†
Ophryotrocha akessoni	0.77	0.74	96	86	8†
Glyceridae	3.28	1.89	58	5	n.a.
Nicomache arwidssoni	6.45	2.07	32	10	84†
Amphisamytha galapagensis	1.77	1.53	86	398	10§
Serpulidae	1.79	1.58	88	25	n.a.

* PETTIBONE (1985) and references cited therein. Range provided is derived from maximum reported sizes of nine species of Polynoidae described from Clam Acres.

† BLAKE (1985).

‡ Range provided is derived from maximum reported sizes of two species of Hesionidae described by BLAKE (1985) from Clam Acres.

§ ZOTTOLI (1983).

n.a. = data are not available.

DISCUSSION

Mollusks, polychaetes, and barnacles recovered from long-term deployments of slate-panel arrays placed at and near hydrothermal vent communities on the Galapagos Rift and East Pacific Rise were almost exclusively post-larval, juvenile and sub-adult stages. This is best documented for populations recovered from Clam Acres, where arrays were *in situ* for more than 3 years. There are several possible explanations for the presence of only post-larval and juvenile stages on the arrays, including the two following, which we argue are unlikely:

Delayed recruitment to slate panels may have occurred because the panels are unnatural surfaces for recruitment at vents. The slate might require a conditioning period before becoming attractive to larvae. But many taxa, especially limpet and clam post-larvae, were recovered from short-term deployments (26 days) of arrays at Clam Acres, and significant recruitment had occurred by 260 days at Rose Garden. This suggests that arrays provided suitable substrate for recruitment within 1 month and definitely within 1 year.

Slow growth rates may account for the predominance of juvenile individuals. Growth rates in the deep sea are thought to be slow (TUREKIAN *et al.*, 1975). But relatively rapid growth rates are estimated for the vent bivalves, *Calyptogena magnifica* (0.27–4.0 cm y^{-1}; TUREKIAN *et al.*, 1983; ROUX *et al.*, 1985) and *Bathymodiolus thermophilus* (1 cm y^{-1}; juvenile growth rates: ~0.1 mm d^{-1}; RHOADS *et al.*, 1981). These growth rates are of the same order as those of shallow water bivalve analogues. The nutrient-rich environment of the vents (RHOADS *et al.*, 1981; SMITH, 1985) may be expected to affect growth rates of ontogenetic stages of bivalves and other vent invertebrates.

If we assume that growth rates of fauna on arrays are relatively rapid and that arrays do not require a lengthy conditioning period before recruitment can take place, then some combination of the following explanations for the immature nature of specimens recovered from recruitment arrays is tenable:

Competition and starvation

The nutritive environment of the arrays may be acceptable for small post-larval and juvenile stages of mollusks and polychaetes, but, as the animals grow, the abundance of appropriate food items may become limiting, leading to competition and starvation. Arrays may only be able to support small individuals.

Migration

Larval stages might recruit to arrays, but as the post-larvae grow, the array becomes an unacceptable habitat. Perhaps it is food-limited, space-limited, or insecure from predation, forcing the juveniles to migrate. Many of the mollusk and polychaete species recovered from the arrays are mobile.

Predation

Predation may be so intense on arrays that recruits are cropped as soon as they are large enough to be detected and consumed, but before they have time to mature. RHOADS *et al.* (1982) suggest that predation activity by the brachyuran crab, *Bythograea thermydron* Williams, is intense on small mussels, although shell deformities inferred to be evidence of predatory activity also can be attributed to effects of heavy metals in vent

effluents (SUNILA and LINDSTROM, 1985). The configuration of the arrays may have promoted predation, inasmuch as the panels were buoyed up off the bottom, putting them in water less toxic to marauding non-vent fish, in addition to crabs, squat lobsters and other invertebrates associated with vents. Predation pressure also may come from macrofauna recruiting to the arrays. Where refuges from predation existed, such as crevices associated with a knot in a polypropylene line, adult individuals of one polychaete species, *Amphisamytha galapagensis*, were found.

We interpret the size distributions of juvenile mollusks and the large variability in lengths of juvenile polychaetes as evidence for intermittent or continuous recruitment in many of the vent-associated species, rather than a single episodic recruitment event as suggested by CORLISS *et al.* (1979) and CRANE and BALLARD (1980). Other evidence for non-episodic recruitment is accumulating, such as J. P. GRASSLE's (1985) analysis of genetic variation in mussel populations at Mussel Bed and Rose Garden. There are factors that might serve to synchronize and punctuate reproductive activity and recruitment of vent species, including systematic fluctuations in the quality and quantity of vent flow. JOHNSON and TUNNICLIFFE (1985), for example, have recorded a semidiurnal fluctuation in water flow over a 6 day period at a Juan de Fuca hydrothermal vent. LITTLE *et al.* (in preparation) document a tidal fluctuation in temperature over a 12 day period at the Guaymas hydrothermal field. A tie between nutrient levels and gamete production in the mussel, *Bathymodiolus thermophilus*, has been documented by SMITH (1985). Three mussels from a peripheral, low-nutrient region of a vent field had undifferentiated gonads (BERG, 1985); evidence for continuous reproductive activity was found within populations of *C. magnifica* and *B. thermophilus* living at vents. It is possible that reproductive activity in these and other species might be punctuated by periods of inactivity, in correspondence with variations in vent flow. Recruitment at vents should in some way reflect this punctuation, but documentation of this phenomenon requires closer monitoring of recruitment and physicochemical parameters through concurrent time-series observations.

Several observations relating to the composition of the fauna collected on recruitment arrays are worth emphasizing:

Protozoans were the most abundant eukaryotes on arrays left in place at Clam Acres for 3.3 years. Thousands of folliculinids covered panels of one array, two species of forams covered panels of the other array. This suggests that protozoans may be significant components of the vent community. The possibility that the forams may harbor chemoautotrophic endosymbiotic microorganisms is being investigated by J. J. LEE (City College of New York).

Ophryotrocha akessoni, a dorvilleid polychaete, recruited in large numbers to arrays. Members of this genus also recruited to colonization experiments in organically enriched soft sediments in non-vent environments (DESBRUYERES *et al.*, 1980; LEVIN and SMITH, 1984).

Spionid polychaetes, abundant on Galapagos arrays, as a taxon are considered to be opportunistic deep-sea fauna (MACIOLEK, 1981; GRASSLE and MORSE-PORTEOUS, 1988).

Copepods and other small, motile crustaceans can be extremely abundant on and around arrays, sometimes forming swarms that can be observed from the submarine.

Despite the occurrence of adult populations of the tubeworm, *Riftia pachyptila* Jones, at all vent sites, no specimens of this species were collected on the recruitment arrays, and the larval stage remains elusive (BERG and VAN DOVER, 1986).

We found a consistent pattern of higher levels of recruitment to slate panel arrays placed within a hydrothermal vent community on the GSC than to arrays placed adjacent to that community. Higher levels of recruitment also were observed on 23–26 day deployments of arrays within the vent field at Clam Acres on the East Pacific Rise than on arrays placed adjacent to the field. Settlement of vent fauna has been suggested to be triggered by unique biological, chemical, and physical conditions associated with the vent habitat (LUTZ *et al.*, 1980). The dominance of vent fauna on 'non-vent' arrays, especially at Galapagos sites (Table 4), raises some questions about the specificity of this triggering mechanism. Nevertheless, it is true that total recruitment is higher in active regions of vents, where conditions are presumably most conducive to settlement and/or survival of vent fauna.

We found different levels of recruitment to arrays at different vent sites on the GSC, with recruitment at Rose Garden > Garden of Eden ≫ Mussel Bed. The hierarchy in estimates of vent flow and standing crop at these vent sites at the time of deployment of arrays was Rose Garden > Mussel Bed > Garden of Eden (HESSLER and SMITHEY, 1983). It is thus difficult to attribute the level of recruitment to a vent as a simple function of either of these characteristics of the vent environment.

Very little is known about locations of source populations or the extent to which supply of larvae to individual vents is independent of proximity to other vents. In an analysis of genetic differentiation in the vent mussel, a species with planktotrophic larvae (LUTZ *et al.*, 1980), GRASSLE (1985) suggests that recruits must come from a small number of genetically distinct populations. Vent communities immediately 'upstream' may serve as sources of propagules 'downstream'. Studies of particulate dispersal (BOLGER *et al.*, 1978), helium isotope distributions (LUPTON and CRAIG, 1981) and local current regimes (LONSDALE, 1977; EDMOND *et al.*, 1982) indicate relatively strong, directional water currents, widespread dispersal of vent emanations, and the potential for widespread dispersal of larvae. Larval stages of vent invertebrates have been collected in the water column immediately overlying vent fields (BERG and VAN DOVER, 1987) and at ~100 m above vent fields (WIEBE *et al.*, 1988). Alternatively, in some species, recruits to vents may be derived primarily from local adult populations associated with a given vent. Non-planktotrophic larval development with relatively low dispersal potential has been inferred from shell and egg morphologies as the typical pattern of development of vent limpets (LUTZ *et al.*, 1984) and the clam *Calyptogena magnifica* (BERG, 1985). These populations may be predominantly self-recruiting, with dependence on dispersal of larvae from nearby vents important in terms of gene flow and colonization of new vent sites but less important in terms of maintenance of populations.

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PART II

TROPHIC STUDIES

Chapter 5

Carbon and Nitrogen Isotopic Compositions of Vent and Seep Symbionts: A Review

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I. Chemical basis for production at deep-sea hydrothermal vents and seeps.

Communities of invertebrates at deep-sea hydrothermal vents and seeps flourish where reduced inorganic compounds serve as energy sources for the chemosynthetic fixation of carbon by procaryotic microorganisms. The microbial basis for life at vents and seeps (reviewed by Jannasch 1985; Jannasch and Mottl 1985; Karl 1987; Prieur et al. 1987; Southward 1987) is largely independent of surface-derived photosynthetic production. Chemosynthetic symbioses of bacteria within tubeworms and mollusks account for the largest portion of total biomass at most known vents and seeps (exceptions are TAG and KANE sites on the Mid-Atlantic Ridge dominated by shrimp; Van Dover et al. 1988). Numerous studies have inferred the net fixation of carbon into reduced organic compounds by endosymbiotic bacteria using energy derived from oxidation of reduced chemical species such as hydrogen sulfide and methane (Cavanaugh et al. 1981; Felbeck et al. 1981; Felbeck 1985; Tuttle 1985; Fiala-Medioni et al. 1986a; Fisher and Childress 1984; Childress et al. 1986; Cavanaugh et al. 1987). Compelling evidence for translocation of chemosynthetically-fixed carbon to host tissues is accumulating, based on ^{14}C-labelling (Felbeck 1985; Fisher et al. 1987; Fiala-Medioni et al. 1986b), ultrastructural studies (Bosch and Grasse 1984; Southward 1982, 1986), and growth-rate experiments (Cary et al. 1988).

Identification of inorganic energy substrates, characterization of metabolic pathways, and demonstration of translocation of organic

material from bacteria to host are three steps toward demonstrating the chemical basis for production at vents and seeps. One of the most promising techniques for rapid, initial assessment of such non-photosynthetic processes is analysis of stable isotopic compositions of symbiont species. The technique is compromised by our inability to interpret carbon and nitrogen sources from isotopic data. While there is no substitute for definitive physiological and biochemical characterization of symbiont and host nutrition, an examination of isotopic compositions of vent and seep symbionts taken together with existing environmental, physiological, morphological, and biochemical data related to these symbionts, will provide a context within which to interpret new data and to formulate hypotheses regarding isotopic compositions and sources of reduced chemical energy and nitrogen. Further, isotopic compositions have the potential to give a synoptic view of symbiont nutrition, providing a time-integrated measure of assimilation within body tissues (Fry and Sherr 1984).

II. Significance of isotopic analyses within the context of tracing origins and transformations of organic material.

Isotopic compositions are expressed in terms of $^{o}/_{oo}$ differences from a standard, where:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3, \text{ and}$$

$$X = {}^{13}C \text{ or } {}^{15}N$$

$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N.$$

Standard reference materials are carbon in the Pee Dee Belemnite (PDB) or nitrogen gas in the atmosphere (AIR). "Lighter" or "more

negative" δ values are enriched in the lighter isotope and reciprocally depleted in the heavier isotope.

Carbon isotopic composition of organic material is a function of the isotopic composition of CO_2 or C_1 precursors to synthesis of organic matter and of enzymatic and substrate limitation fractionation effects that take place during fixation of CO_2 or C_1 compounds (reviewed for photosynthesis in Fry and Sherr, 1984; O'Leary 1988). There is, in general, a discrimination against the heavier, ^{13}C isotope during fixation such that organic material formed is isotopically light. In plants, the amount of discrimination relative to the original carbon substrate is dependent on the fixation pathway: on average, C_3 plants are isotopically lighter than CO_2 by 23°/oo while C_4 plants are lighter by only 7°/oo (O'Leary 1988; based on 1000 analyses). Isotopic discrimination in chemoautotrophic bacteria is less well-known: Ruby et al. (1987) found a 25 °/oo fractionation toward lighter $\delta^{13}C$ values in two laboratory-isolated species of sulfide-oxidizing chemoautotrophic bacteria; Zyakun et al. (1981; cited in Fry and Sherr 1984) report a 10 to 20 °/oo carbon isotopic fractionation in methane-oxidizing chemoautotrophic bacteria. Van Dover and Fry (Submitted) infer heavy $\delta^{13}C$ values (> -11°/oo) for *in situ* populations of free-living chemoautotrophic bacteria at several vent sites. Isotopic variations introduced at the level of fixation are typically passed on through metabolic and trophic transformations with little or no alteration (reviewed in Fry and Sherr 1984; Peterson and Fry 1987), i.e., consumer tissues reflect the average $\delta^{13}C$ ratio of their diet.

Nitrogen isotopic composition of organic material is a function of the isotopic composition of the inorganic precursor (N_2, NO_3^-, NH_4^+) and of substrate limitation. Isotopically light nitrogen is preferentially excreted by consumers, resulting in reciprocally heavy consumer tissues (Minagawa and Wada 1984); a systematic, 2-4°/oo increase in $\delta^{15}N$ is associated with a single trophic step (Wada et al. 1987).

III. Isotopic composition of vent symbionts.

Although vent communities have been discovered in a variety of geographically and geologically distinct areas, isotopic compositions of vent symbionts are published for only three species that live on the Galapagos Spreading Center and at Clam Acres (21°N) on the East Pacific Rise, namely the tubeworm *Riftia pachyptila* and the bivalve mollusks *Bathymodiolus thermophilus* and *Calyptogena magnifica*. Van Dover and Fry (Submitted) provide data on three symbiont species occurring at Marianas Back Arc Basin and Hanging Gardens (21°N) vent sites. Geographic locations of vent sites are plotted in Figure 1. Isotopic data are summarized in Table 1 and Figure 2. In a separate paper, Van Dover and Fry (In preparation) discuss patterns in isotopic compositions of additional vent symbiont species from Gorda Ridge, Juan de Fuca Ridge, Guaymas Basin, and 11°N on the East Pacific Rise.

A. EARLY WORK OF RAU, WILLIAMS, AND COLLEAGUES (1979-1985)

Rau and Hedges (1979) were the first to suggest a non-photosynthetic food source for vent symbionts based on isotopic evidence. They compared $1\delta^{13}C$ values of the mytilid mussel, *Bathymodiolus thermophilus* from a Galapagos hydrothermal vent with shallow-water *Mytilus californianus* and *M. edulis* and found strikingly lower values in the vent mussel mantle and foot tissues (average = -33.2°/oo) than in *Mytilus*. Since *Mytilus* values were within the range for marine organisms using photosynthetically-derived organic material, Rau and Hedges concluded that the mussels themselves could not be responsible for fractionation of carbon to an unusual extent. The ^{13}C-depleted tissues of *B. thermophilus* in comparison with other marine organic carbon were thus taken as evidence for a non-photosynthetic primary food source.

Rau (1981a) expanded the data base on carbon isotopic compositions of vent symbionts with publication of $\delta^{13}C$ values for the tubeworm, *Riftia pachyptila*, and the clam, *Calyptogena magnifica*, from Galapagos and East Pacific Rise vents. Clam $\delta^{13}C$ values near -32°/oo matched those of the mussel from the same area. Rau eliminated an alternative hypothesis of pressure or depth effects on carbon isotopic composition of clams and mussels by noting that $\delta^{13}C$ values of bathypelagic organisms taken from depths comparable to the vents were usually similar to $\delta^{13}C$ values of surface-derived organic material (Williams and Gordon 1970; Eadie and Jeffrey 1973). Vent clams and mussels were clearly unique in their isotopic composition relative to previously known invertebrates and thus were using an isotopically different food source. In a discussion of this food

source, Rau cites the work of Degens et al. (1968), Degens (1969), and Fuchs et al. (1979) as evidence for 30°/oo fractionation effects in chemoautotrophic bacteria and concludes that the 28°/oo difference between bivalve $\delta^{13}C$ and the $\delta^{13}C$ of vent water ($\geq$4°/oo; Craig et al. 1980) can be explained by chemosynthesis of organic matter and subsequent preservation of isotopic abundances in higher trophic level biomass.

Isotopic composition of tubeworms turned out to be more enigmatic. *R. pachyptila* tissues were relatively heavy in terms of carbon isotopic composition, with $\delta^{13}C$ values around -11°/oo. Acid-treatment of tubeworm tissues prior to analysis eliminated the possibility of ^{13}C-rich carbonate contamination. Rau noted the similarity between tubeworm $\delta^{13}C$ values and $\delta^{13}C$ values of shallow-water marine animals dependent on C_4 plant material, but the lack of a crucial C_4 enzyme (phosphoenolpyruvate carboxylase; Felbeck 1981) in this species seemed to preclude a C_4 pathway. Instead, Rau proposed that a limited internal supply of CO_2 relative to internal demand for carbon fixation by bacterial chemoautotrophic endosymbionts could explain the high $\delta^{13}C$ values observed in *Riftia pachyptila*. Rau's 1981a paper was published as a companion paper to Cavanaugh et al. (1981) and Felbeck et al. (1981) wherein chemoautotrophic endosymbioses in tubeworms were first described; the bivalves were initially presumed to be filter-feeders.

The discrepancy between tubeworm and bivalve $\delta^{13}C$ values was confirmed by Williams et al. (1981). In addition, using analysis of ^{14}C activity in tubeworm and mussel tissue, these authors showed that the principal source of dietary carbon for both species was dissolved

inorganic carbon in vent effluent waters derived from both magmatic and ambient bottom water. Identical ^{14}C activities in tubeworm and mussel tissue implied that both species used the same inorganic carbon source, despite the 23 °/oo difference in their $\delta^{13}C$ values. Williams et al. considered the possibility of magmatic methane as a primary carbon source for the mussel: since magmatic methane has no radiocarbon activity, if it were the sole carbon source for synthesis of organic carbon used by the mussel, mussel tissue would have no measurable radiocarbon activity; such was not the case. Williams et al. calculate that the mussel incorporates 13% 'dead' CO_2 of magmatic origin into its tissues and that <25% of the dietary carbon obtained by the mussel could be from surface-derived organic carbon; the remainder of the carbon must be from ambient bottom water.

Rau (1981b) published the first data on nitrogen isotopic compositions of three species of vent fauna using material collected at Galapagos vent sites. $\delta^{15}N$ values increased as a function of each species' assumed trophic level, corroborating earlier observations (Wada et al. 1987) within shallow-water systems that trophic processes tend to increase $\delta^{15}N$ values in resultant consumer tissue. Furthermore, $\delta^{15}N$ values of vent primary producers and primary consumers (primary producers: tubeworm trophosome, $\delta^{15}N$ = +1.8; primary consumers: tubeworm vestimentum and clam mantle, $\delta^{15}N$ = +2 to +4.9 °/oo) were more negative than deep-ocean sedimentary organic nitrogen (+5 to +13 °/oo; Sweeny and Kaplan 1980, among others cited in Rau 1981b), implying that assimilated nitrogen within the vent community had undergone little recycling and was derived from a local inorganic source. Since $\delta^{15}N$ values of such inorganic sources are

not known for vent waters, Rau is left to speculate on the pathway of nitrogen assimilation. One option is local N_2 fixation: the $\delta^{15}N$ of dissolved N_2 in the deep-ocean is low, between +0 and +2 °/oo, and little isotopic fractionation is known to occur during fixation of N_2 by autotrophs (Kohl and Shearer 1980, among others cited by Rau 1981b); tubeworm and clam $\delta^{15}N$ values of +1.8 to +4.9 are consistent with this hypothesis. On the other hand, Rau cites examples of large isotopic fractionations during nitrate and ammonium assimilation in autotrophs under conditions of elevated nutrient concentrations (Wada and Hattori 1978; Kohl and Shearer 1980) which could account for depleted ^{15}N values in vent species.

In a 1985 review, Rau considered the isotopic compositions of vent species within the context of developments in other areas. In particular, Rau (1985) noted that the similarity in $\delta^{13}C$ values observed in two species of mollusks at two geographically separated vents compared to the significant isotopic variability of dissolved inorganic carbon (DIC) at the vents (0 to -7°/oo; Craig et al. 1980) argued for occupation of very specific sites in or around vents with access to isotopically uniform DIC. But Craig's values are calculated end-member values (i.e., undiluted 350°C effluents). Diluted vent water in which vent fauna thrive is likely to be isotopically indistinct from the surrounding ambient seawater. Rau also suggested that *Lamellibrachia* sp., a smaller, non-vent relative of *Riftia pachyptila*, had more negative $\delta^{13}C$ values ($\delta^{13}C$ values not given) than *Riftia* because carbon would be less limiting in smaller individuals. Heavy carbon isotopic compositions of small species of vent vestimentiferans negate this hypothesis (e.g. *Oasisia alvinae*

from Hanging Gardens: $\delta^{13}C$ = -11.4°/oo; Van Dover and Fry, Submitted).

B. RECENT STUDIES BY FISHER AND COLLEAGUES AT ROSE GARDEN, GALAPAGOS SPREADING CENTER

Between 1981 and 1988, symbiotic associations within gill tissues of vent clams and mussels were established (Cavanaugh et al. 1981; Felbeck 1981; Fiala-Medioni 1984) but no new data on isotopic compositions of vent symbionts were published. In 1988, Fisher and no fewer than 17 collaborators (Fisher et al., 1988a,b,c) published a series of papers on physiological and biochemical variation in tubeworm, clam and mussel populations at the Rose Garden vent site on the Galapagos Spreading Center in 1985. These papers included relatively extensive data sets on isotopic composition of the three symbiont species. Fisher's general premise is that microhabitat variation in geochemical flux within the vent field is correlated with species distributions. Furthermore, a given species may be forced to occupy a less than optimal microhabitat; variability in physiological and biochemical parameters, including isotopic composition, could serve as a sensitive index to the degree of freedom each species has in tolerating suboptimal conditions.

i) *Riftia pachyptila*

Within populations of *Riftia pachyptila*, Fisher et al. (1988a) found very little variation within bulk trophosome tissue in either $\delta^{13}C$ (-10.9$\pm$ 0.2 o/oo; n=25) or $\delta^{15}N$ (+1.8$\pm$0.2 °/oo; n=13). Within individual trophosomes, $\delta^{13}C$ values spanned as much as 2.2 °/oo; this variability was attributed to either sampling error or to an

undetermined variability in metabolic characteristics within different regions of the trophosome. Other physiological parameters, such as activity of ATP sulfurylase and sulfide oxidase and concentrations of elemental sulfur, water and extractable lipid contents of trophosome, also varied within different regions of the trophosome (Fisher et al. 1988a). De Burgh (1986) presented evidence for anterior-to-posterior gradients in bacterial size and chemistry (including sulfur, zinc, and other in elements) within the trophosome of related tubeworm species, *Ridgea piscesae* and *R. phaeophiale*.

Fisher et al. (1988a) argue strongly for uptake of nitrate by tubeworms, based on enzymatic, biogeochemical, and isotopic data: nitrate reductase activity has been demonstrated in *Riftia* trophosome (Felbeck 1981), vent organisms are implicated in the removal of nitrate from venting water (Johnson et al. 1988), and average $\delta^{15}N$ values for tubeworms are about 8 °/oo heavier than $\delta^{15}N$ values of mussels suspected of assimilating organic nitrogen originating from N_2 (Fisher et al. 1988b).

$\delta^{15}N$ values of trophosome tissues were, on average, 3 °/oo lighter than non-trophosome tissues (n=2). This difference is consistent with a single trophic step between symbionts and host and was also observed by Rau (1981b).

ii) ***Bathymodiolus thermophilus***

Of the three vent symbiont species, carbon isotopic composition was most variable in mussel tissues (Fisher et al. 1988b), ranging from -30.5 to -37.1 °/oo (n=31). Mussels collected from central clumps, near the greatest flux of hydrothermal effluent, had the most negative $\delta^{13}C$ values; peripheral populations were more positive.

Some of the variation in mussel carbon isotopic composition might be accounted for by occupation of sites at vents with isotopically distinct DIC. Fisher et al. (1988b) estimate the maximum variability in $\delta^{13}C$ values of DIC at Rose Garden, based on the 350°C end-member $\delta^{13}C$ value, and find a small range (0.14 °/oo) that cannot account for the variation in $\delta^{13}C$ values of mussel tissues.

Since mussels have a functional filter-feeding apparatus and digestive system (LePennec and Hily 1984; LePennec and Prieur 1984), Fisher et al. present the argument that variability in mussel $\delta^{13}C$ is likely to reflect a greater contribution of particulate organic carbon (POC) to the nutrition of peripheral mussel populations. At vent sites on the East Pacific Rise and Marianas Back Arc Spreading Center, Van Dover and Fry (submitted) infer $\delta^{13}C$ values of -11 to -15 °/oo for POC. If these values apply to Rose Garden POC, then, using a simple mixing model, where

$$\delta^{13}C_{mussel} = (f)\delta^{13}C_{symbiont} + (1-f)\delta^{13}C_{POC},$$

the maximum contribution of POC (-15 o/oo) to a peripheral, -30.5 °/oo mussel with -37.1 °/oo symbionts would be 30%. If, as Fisher et al. suggest, Rose Garden POC has $\delta^{13}C$ values matching those of endosymbionts, such a simple mixing model would be inappropriate.

In pursuing lines of evidence in support of non-symbiont contributions to nutrition of mussels, Fisher et al. note differences between $\delta^{13}C$ values of gill and non-gill tissues in the mussel: gill tissue is consistently more negative by about 1 °/oo. Fisher et al. argue this relationship should be the reverse if symbiont carbon accounted for 100% of the mussel nutrition since "animals normally

discriminate against ^{13}C during assimilation of food (Rau and Hedges 1979)". On the other hand, selective assimilation of ^{13}C-enriched components in natural diets has been demonstrated by Fry et al. (1984). There is also the possibility of preferential loss of the lighter isotope during respiration (DeNiro and Epstein 1978) by the host tissue. Recent work by Fiala-Medioni and Le Pennec (1987) shows that gill tissue of vent mussels and clams are rich in lipids; lipid synthesis involves a discrimination against the heavy carbon isotope, resulting in light $\delta^{13}C$ values relative to protein-rich muscle tissue (Parker 1964; Van Der Merwe 1982). These points suggest that additional analyses and experiments must be undertaken before the difference in isotopic composition between gill and non-gill tissue can be interpreted as conclusive evidence for non-symbiont contributions to mussel nutrition.

A very striking difference in $\delta^{15}N$ values of central (-3.9 °/oo; n=14) and peripheral (+3.5 °/oo; n=18) mussel populations is reported by Fisher et al. (1988b). Two alternative hypotheses for this variability are presented:

i) Nitrogen assimilation in mussels is mediated entirely through translocation from endosymbionts: Where inorganic nitrate or ammonium is unlimiting, $\delta^{15}N$ values of mussel tissue should reflect endosymbiont assimilation of >+6 °/oo deep-ocean NO_3^- or NH_4^+ with little fractionation (Wada and Hattori 1978; Macko et al. 1987). Where concentrations of NO_3^- of NH_4^+ are low, as within the central mussel populations at Rose Garden (Johnson et al. 1988), N_2 fixation by endosymbionts might occur. Negative $\delta^{15}N$ values that reflect the 0 °/oo of deep-ocean N_2 and the -3 °/oo fractionation effect of

nitrogen fixation (Macko et al. 1987) might then be expected in mussels occurring under these conditions.

ii) Nitrogen assimilation in mussels is mediated through ingestion of particulate organic nitrogen and translocation from endosymbionts: Fisher et al. suggest that $\delta^{15}N$ values of PON vary between sites, with peripheral PON $\delta^{15}N$ values between 2 and 20 °/oo, reflecting a greater contribution of normal oceanic nitrogen sources (Rau 1981b; Paull et al. 1985) and with central PON $\delta^{15}N$ values less than 0 °/oo, reflecting N_2 fixation by free-living microorganisms. Site-specific differences in PON $\delta^{15}N$ and symbiont N_2 fixation would account for observed site differences in mussel $\delta^{15}N$.

Fisher et al. (1988b) found no consistent pattern in $\delta^{15}N$ values in paired samples of gill and non-gill tissue of mussels (in contrast to data above on *Riftia pachyptila* trophosome and non-trophosome tissues and data below on *Calyptogena magnifica* gill and non-gill tissues). This suggests that the nitrogen isotopic relationship between bacterial symbionts and host tissues in mussels may be compounded by variable host dependency on particulate organic nitrogen.

iii) *Calyptogena magnifica*

$\delta^{13}C$ values for clam gill and non-gill tissues ranged from -34.4 to -31.4 °/oo (n=27) in Rose Garden populations (Fisher et al. 1988c), with gill tissue slightly (0.6 °/oo) but significantly more negative than non-gill tissue.

$\delta^{15}N$ values for clam gill tissues averaged +1.8 °/oo (n=10) and for non-gill tissues averaged +4.5 °/oo (n=10). The average 2.7 °/oo

(n=10) difference between gill and non-gill tissue is consistent with a single trophic step between symbionts and host.

Carbon and nitrogen isotopic composition in clams varied significantly with microhabitat at Rose Garden in one instance. Fisher et al. suggest this reflects a varied contribution of particulate organic material.

C. SUMMARY OF MAIN OBSERVATIONS BY FISHER et al. 1988 a,b,c

* Carbon and nitrogen isotopic compositions of tubeworms, clams and mussels at Rose Garden are similar to values reported by earlier investigators.

* Significant variations in $\delta^{13}C$ within individual trophosome tissues of tubeworms were observed but not explained.

* Isotopic compositions of tubeworm and clam populations showed little variability, consistent with their restricted distributions to specific microhabitats within the vent field.

* Significant variations in $\delta^{13}C$ and $\delta^{15}N$ values of mussel tissues were correlated with microhabitat variation; this isotopic variation may reflect a combination of site-specific variation in $\delta^{13}C$ and $\delta^{15}N$ values of inorganic nutrients and of site-specific variation in ingestion of particulate organic material by mussels.

* A consistent increase in $\delta^{15}N$ between symbiont and host tissues argues for a simple producer-consumer relationship in tubeworms and clams; variation in $\delta^{15}N$ relationships between symbiont and host tissues in mussels is consistent with the hypothesis of variable contributions of particulate organic material to mussel nutrition.

* Tubeworms may derive their nitrogen through nitrate assimilation by symbionts; a combination of nitrate assimilation, N_2-fixation and particulate organic nitrogen uptake may apply in the mussel; sources of nitrogen for clams are ambiguous.

D. ISOTOPIC DATA ON VENT SYMBIONTS FROM HANGING GARDENS (21°N, EAST PACIFIC RISE) AND MARIANAS BACK ARC BASIN.

Recent work by Van Dover and Fry (Submitted) extend the isotopic data set for vent fauna both faunistically and geographically. Two species of vent tubeworms (*Riftia pachyptila, Oasisia alvinae*) from the Hanging Gardens vent site (Berg and Van Dover 1987) were analyzed. Both species had $\delta^{13}C$ values near -11 °/oo. At Marianas vents in the western Pacific, a symbiont-bearing coiled gastropod (*Alviniconcha hessleri*) is a dominant component of the faunal community. Gastropod gill tissues hosting symbiotic bacteria (Stein 1988) averaged -28.9°/oo; muscle tissue averaged -27.9°/oo. These values are more than 4°/oo heavier than values observed in vent bivalves with symbionts, including the Marianas mussel (gill tissue = -34.8; muscle tissue = -32.8). Van Dover and Fry suggest that the gastropod may have a mixed diet of translocated symbiont organic material ($\delta^{13}C$ = ~-35°/oo) and grazed free-living microorganisms ($\delta^{13}C$ = ~-11°/oo).

Nitrogen isotopic compositions of Hanging Gardens tubeworms were comparable to those of Galapagos vent tubeworms (between +1.8 and +4.5). $\delta^{15}N$ values of the hairy gastropod were slightly heavier by 1 or 2 °/oo than those of vent clams or mussels from Clam Acres and

Galapagos sites (Van Dover and Fry, Submitted). This enrichment in ^{15}N is consistent with a mixed diet in the snail via translocation and grazing. The Marianas mussel was significantly depleted in ^{15}N (gill = -34.8°/oo; muscle = -0.5°/oo) relative to all other analyzed components of the Marianas food web, but falls within the range of nitrogen isotopic compositions measured in *Calyptogena magnifica* and *Bathymodiolus thermophilus* from eastern Pacific vent sites (Van Dover and Fry, Submitted).

IV. Isotopic composition of seep symbionts.

The first report of seep communities in the deep sea appeared in the literature in 1984 (Paull et al. 1984) with the description of a community in the Gulf of Mexico comprised of fauna closely resembling vent-type taxa, including vestimentiferan tubeworms, mytilid mussels, and vesicomyid clams. Since 1985, seep communities have been discovered elsewhere in the Gulf of Mexico (Kennicutt et al. 1985), off Oregon (Seuss et al. 1985; Kulm et al. 1986) and Northern California (Kennicutt et al. in press), off Honshu, Japan (Le Pichon et al. 1987; Ohta and Laubier 1987;), and, most recently, off Nova Scotia (Mayer et al. 1988). Isotopic data has been used to infer non-photosynthetic sources of nutrition for populations of tubeworms and bivalves occurring at seeps. Geographic locations of seep sites are plotted in Figure 1. Isotopic data are summarized in Table 1 and Figure 2.

A. FLORIDA ESCARPMENT, GULF OF MEXICO

Abundant populations of vestimentiferan tubeworms (*Escarpia laminata*) and mussels (*Bathymodiolus* sp.) occur where hypersaline porewater at ambient temperature seeps from the contact between the limestone platform of the Florida Escarpment and the hemipelagic sediments of the abyssal Gulf of Mexico (Paull et al. 1984). Paull et al. (1985) report highly fractionated carbon isotopic compositions in mussels ($\delta^{13}C$ = -74.3±2.0 o/oo, n=10) and suggest that the mussel derives its carbon from recent biogenic methane ($\delta^{13}C$ = -60 to -90 °/oo; 60% modern) via translocation from endosymbiotic methanotrophic bacteria. Similar $\delta^{13}C$ values (-72 °/oo) for Florida seep mussels are reported by Cary et al. (submitted); these authors note that sediment methane $\delta^{13}C$ at the Florida Seeps is -80 to -90 °/oo (analyzed by J. Chanton, University of North Carolina) and account for the 8-18 °/oo discrepancy between $\delta^{13}C$ values of mussel tissue and source methane by two plausible scenarios: i) microbial consumption of biogenic methane at the sediment-water interface, leading to ^{13}C enrichment of residual methane (Zyakun et al. 1981) available to symbionts, or ii) a contribution of isotopically "heavy" particulate material to mussel $\delta^{13}C$ through filter-feeding. Cavanaugh et al. (1987) support the hypothesis of symbiotic methanotrophic bacteria with ultrastructural evidence for the presence of "type 1" methane-oxidizing bacteria within gill tissue of mussels and bioassay evidence for the activity of enzymes (methanol dehydrogenase, hexulose phosphate synthase) diagnostic of methane oxidation and the absence of the enzyme (ribulose bisphosphate carboxylase (RUBPCase)) diagnostic of autotrophic CO_2 fixation. Cary

et al. (Submitted) also report methanol dehydrogenase activity in Florida Seep mussels.

Tubeworm isotopic compositions ($\delta^{13}C$ = -42.7±0.7 o/oo, n=3; $\delta^{15}N$ = -2.8 o/oo, n=2) similar to those of bivalves at hydrothermal vents led Paull et al. (1985) to infer a similar biochemical pathway, namely CO_2 fixation by sulfide-oxidizing endosymbiotic bacteria and subsequent translocation to host tissues. Equally light $\delta^{13}C$ composition of tube worms tissues (trophosome: -47.2±2.4 °/oo, n=4; vestimentum: -44.8±3.5 °/oo, n=3) are reported by Cary et al. (submitted), along with significant activity of RUBPCase and ATP sulfurylase enzymes diagnostic of sulfur-based chemoautotrophy in trophosome tissues. Large deposits of elemental sulfur in the trophosome, high levels of sulfide oxidase activity, and circulating sulfide binding proteins are further evidence that *Escarpia laminata* relies on a sulfur-based endosymbiosis for nutrition (Cary et al. 1987).

A vesicomyid clam, *Calyptogena* sp., also occurs at the Florida seeps. $\delta^{13}C$ values of -36.7 °/oo (n=3) suggest that it, too, relies on sulfur-based CO_2 fixation (Cary et al. submitted).

Nitrogen isotopic composition of Florida seep tubeworms (-2.8 °/oo; n=2) and mussels (-8.9 °/oo; n=2) are light relative to particulate organic nitrogen and indicate assimilation of inorganic nitrogen mediated by chemosynthetic bacteria (Paull et al. 1985).

B. LOUISIANA SEEPS, GULF of MEXICO

In 1985, Kennicutt et al. reported dense biological communities, including vestimentiferan tubeworms (*Lamellibrachia* sp.) and

vesicomyid clams (*Calyptogena ponderosa*), associated with regions of oil and gas seepage on the Louisiana continental slope (600-700 m). Carbon isotopic compositions of tubeworms ($\delta^{13}C$ = -27.0 °/oo) and clams ($\delta^{13}C$ = -35.4 °/oo) were cited as evidence for chemosynthetic production at these hydrocarbon seep sites. Brooks et al. (1987) present ultrastructural evidence for symbiotic bacteria in the lucinid clam *Pseudomiltha* sp., the vesicomyid clams *Vesicomya cordata* and *Calyptogena ponderosa*, the vestimentiferan tubeworm *Lamellibrachia* sp. and an undescribed species of tubeworm; RUBPCase, ATP sulfurylase and adenosine-5'-phosphosulfate reductase activities and elevated elemental sulfur content of both vestimentiferans and the lucinid clam provide further indications of sulfide-based chemoautotrophic endosymbiotic associations in these species. $\delta^{13}C$ values of the three bivalve species ranged between -30 and -42 °/oo and are characteristic of clams with sulfur-based bacterial symbionts (Brooks et al. 1987). $\delta^{13}C$ values of tubeworm tissues are given for *Lamellibrachia* sp. (-27 °/oo, -43.2 o/oo) and an unidentified, *Escarpia*-like species (-30.4 to -40.9 °/oo; n=3). Brooks et al. report a range of -20 to -58 °/oo for combined tubeworm tissues and tubes. In a separate paper, MacDonald et al. report $\delta^{13}C$ values of -21.1, -24.3, and -23.4°/oo for "vascular" tissues of 3 lamellibrachiid tubeworms from the Bush Hill site. Trophosome tissue of the escarpiid tubeworm had a $\delta^{13}C$ value of -27.4°/oo. Brooks et al. suggest that values lighter than -42 °/oo in tubeworms might reflect a contribution from methane-oxidizing endosymbionts in vestimentiferans, although Arp et al. (1986) and Childress et al. (1984) suggest that methanotrophic symbioses are unlikely in

tubeworms because of the difficulties in transporting sufficient methane through the vascular system to the trophosome.

Several lines of evidence suggest that the mussel found at Louisiana seeps, like its relative at Florida seeps, relies on methane-oxidizing endosymbiotic bacteria for its nutrition: Brooks et al. (1987) measured methanol dehydrogenase activity in the mytilid mussel and describe ultrastructural evidence for bacterial symbionts with stacked internal membranes typical of methanotrophs in mussel gill tissue. Mytilid $\delta^{13}C$ values of -40.1 to -57.6 °/oo (Brooks et al. 1987) may reflect $\delta^{13}C$ values of thermogenic methane (> -45 °/oo; Bernard et al. 1987, cited in Brooks et al. 1987) in its environment. Childress et al. (1986) demonstrated consumption of methane by gills of the Louisiana seep mussel and give $\delta^{13}C$ values for gill tissue of -51.9 °/oo (n=3) and $\delta^{13}C$ for mantle tissue of -54.7 (n=3). ^{14}C data on mussels and other seep fauna at Louisiana sites indicate that organic carbon within the community is principally derived from either direct use of fossil (or "dead") methane by mussels or through extensive biodegradation of oil and gas (with active CO_2 production) by microorganisms.

$\delta^{15}N$ values for Louisiana seep fauna (Brooks et al. 1987) are variable, both within species (e.g., -12.9 to +3.0 °/oo in the mussel) and within the community (range of values measured for the four bivalves and two tubeworms listed above was -12.9 to +7.1 °/oo). In the absence of high concentrations of NO_3^- and NH_4^+ at Louisiana seeps, Brooks et al. suggest that the most likely source of nitrogen within the community is fixation of N_2 gas. N_2 gas isolated from a nearby oil well had a $\delta^{15}N$ value of -2.9 °/oo; biochemical

fractionation during fixation of this gas would result in organic nitrogen near -6 °/oo. Natural gases can be as depleted as -14.6 °/oo (Stahl et al. 1977). Variability in $\delta^{15}N$ values of N_2 gas in the immediate vicinity of animal populations could explain the range and variability in $\delta^{15}N$ values of seep symbionts.

C. OREGON SUBDUCTION ZONE

Clam and tubeworm populations associated with rock outcrops of the Oregon subduction zone and seeping fluids at 2306 m are described by Kulm et al. (1986). These authors found the $\delta^{13}C$ value of clam (*Calyptogena* sp.) gill tissue to be -51.6 °/oo and the $\delta^{13}C$ value of tubeworm (*Lamellibrachia barhami*) tissue to be -31.9 °/oo. Lack of evidence for elevated hydrogen sulfide concentrations in overlying water, detection of methane-enriched fluids at one site, and light carbon isotopic compositions of clam and tubeworms tissues relative to those of hydrothermal vent clams and tubeworms led Kulm et al. to speculate that subduction zone clams and tubeworms host methane-oxidizing endosymbionts.

D. JAPAN TRENCH SUBDUCTION ZONE AND SAGAMI BAY

French and Japanese scientists discovered dense benthic communities dominated by several species of vesicomyid clams in Nankai Trough (1000 m), Japan Trench (5960 m), and Sagami Bay (1170 m) (Cadet et al. 1987; Ohta and Laubier 1987). Vestimentiferan tubeworms were also encountered near the Sagami Bay site (Okutani and Egawa 1986; Ohta et al. 1987; Hashimoto et al. In Press). Geochemistry of the sites is outlined in Sakai et al. (1987). Boulegue et al. (1987a)

report ^{13}C-enriched isotopic compositions of *Calyptogena* sp. ($\delta^{13}C$ values for gill: -40.1 °/oo; for mantle: -38.1 °/oo; n=1) from the Japan Trench site. The authors propose that oxidation of thermogenic methane advected from pore waters could be mediated by endosymbiotic bacteria observed within gill tissue of the bivalves; elevated sulfur content of gill tissue suggests that hydrogen sulfide from advected porewaters must also be considered as a possible energy source for chemosynthetic processes. Saino and Ohta (In Press) present similar $\delta^{13}C$ data (gill: -38.7 °/oo; mantle: -36.7 °/oo; n=1) for *Calyptogena phaseoliformis* from the Japan Trench and for *C. soyoae* from Sagami Bay (gill: -35.3 °/oo; mantle: -35.0 o/oo; n=1). High concentrations of methane in seawater and elemental sulfur in sediment are associated with the clam beds at the Sagami Bay site; both the methane and the sulfur are thought to be of biological origin (Saino and Ohta 1987). In their discussion, Saino and Ohta identify three potential sources of carbon for primary production: deep-ocean CO_2 (0 °/oo), porewater CO_2, methane; porewater CO_2 is derived either from decomposition of organic material or from oxidation of methane. Endosymbiotic bacteria hosted by *Calyptogena phaseoliformis* do not show stacks of intracytoplasmic membranes typical of type I methanotrophs (Fiala-Medioni and Le Pennec 1987) and found in known methanotrophic symbioses (Childress et al. 1986; Cavanaugh et al. 1987); furthermore, there are abundant of elemental sulfur crystals in gill tissue (Fiala-Medioni and Le Pennec 1987) of this species. Fiala-Medioni and Le Pennec (1988) conclude that active sulfur metabolism occurs in *C. phaseoliformis*. Without isotopic values for porewater methane and CO_2, it is not possible to identify the carbon

source. $\delta^{13}C$ value for trophosome of the vestimentiferan tubeworm *Lamellibrachia* sp. was -25.8 °/oo (Saino and Ohta In Press).

$\delta^{15}N$ values for bivalves from Japan seeps were variable, ranging from -9.6 °/oo (n=1) in gills of *C. soyoae* to -3.3 °/oo (n=1) in gills of *C. phaseoliformis* to -2.4 °/oo in gills of *Calyptogena* sp. (Boulegue et al. 1987a; Saino and Ohta In Press). Boulegue et al. note a 6 °/oo shift toward the heavier isotope in $\delta^{15}N$ values from gill to mantle tissue reflecting a food chain enrichment in ^{15}N. These authors also suggest that N_2 fixation occurs in gills of bivalves and support this hypothesis by noting anomalously high molybdenum content of gill tissue which might reflect the presence of molybdenum enzymes obligate in N_2 fixation. Saino and Ohta (In Press) argue that ^{15}N-depletion in symbiont tissues is too great to be accounted for by N^2 fixation. They suggest that assimilation of isotopically light ammonium derived from biological ammonification of organic nitrogen may also contribute to observed $\delta^{15}N$ values. *Lamellibrachia* sp. trophosome $\delta^{15}N$ was -4.1 °/oo (n=1; Saino and Ohta In Press).

E. NORTHERN CALIFORNIA CONTINENTAL SLOPE

The presence of gas hydrates in shallow cores from the continental slope off northern California (Field and Kvenvolden 1985) led Kennicutt et al. (Submitted) to dredge for biological specimens in the area. Dredge hauls contained live *Vesicomya* sp. (formerly identified as *Calyptogena pacifica*; R. Turner, pers. comm.), a species known from hydrothermal vents in Guaymas Basin (Grassle 1986). Large amounts of elemental sulfur, together with $\delta^{13}C$ values

of -36.0 °/oo in gill tissues of this bivalve lead the authors to conclude that sulfide-oxidizing endosymbionts fix CO_2 within the gills. $\delta^{15}N$ values of clam tissues ranged from +0.9 to +1.8 °/oo, indicating a local rather than sedimentary or pelagic source of nitrogen.

F. LAURENTIAN FAN, NOVA SCOTIA

Mayer et al. (1988) describe dense communities of vesicomyid bivalves on Laurentian Fan turbidite deposits in a passive margin environment. In considering potential energy sources, Mayer and his colleagues did not detect any signs of fluid flux from the sediments, but they do cite elevated H_2S values in one push core from the site; they also note the presence of hydrocarbon and methane-rich sediments in nearby regions (Piper et al. 1984). Reduced compounds fueling chemosynthetic production at this site are presumably derived from older, organic-rich fan sediments exposed by the 1929 Grand Banks earthquake. Boulegue et al. (1987b) interpret results of isotopic analysis of clam shells ($\delta^{13}C$ = -1 to -6 °/oo) as evidence for a thermogenic methane source of carbon. Van Dover and Fry (unpublished) analyzed foot and mantle tissues of *Calyptogena* sp. that dominate communities on the Laurentian Fan: $\delta^{13}C$ = -34.7 °/oo; $\delta^{15}N$ = -1.8 °/oo.

V. Summary and Prospectus

Over the past ten years, a variety of symbiont-bearing species from diverse deep-sea environments have been analyzed for their

carbon and nitrogen isotopic compositions. Based on interpretations of these analyses made by the authors cited above, the following general observations may be made:

* Carbon and nitrogen isotopic compositions of vent symbiont species are distinctive compared to isotopic compositions of non-vent deep-sea invertebrates. This observation is consistent with the hypothesis of trophic dependence on local sources of primary nutrients and with the concept of chemosynthetic communities.

* Within vent symbiont species, there is a dichotomous distribution of carbon isotopic compositions, with mollusk species (3 spp) having $\delta^{13}C$ values < -27°/oo and tubeworm species (2 spp) having $\delta^{13}C$ values > -12°/oo. Isotopically light carbon values associated with vent bivalves are attributed to isotopic discrimination during CO_2 fixation via a C_3-like biochemical pathway in autotrophic endosymbionts. Isotopically heavy carbon values associated with vent tubeworm tissues are attributed to CO_2-limitation, possibly through a C_4-like CO_2 fixation pathway.

* Sources of nitrogen for vent symbionts are difficult to interpret from isotopic compositions alone. Nitrate assimilation, N_2 -fixation and, for some symbiont species, particulate organic nitrogen uptake, are all potential sources.

* Isotopic compositions of animal tissues have been used to infer

non-photosynthetic sources of nutrition for populations of seep bivalves and tubeworms.

* Extremely light carbon isotopic compositions of some seep symbiont species are consistent with their dependence on isotopically light biogenic or thermogenic methane as a carbon source rather than seawater DIC.

* Unlike vent tubeworms, seep tubeworms have isotopic compositions that are relatively light.

* Variability in isotopic compositions within and among populations of vent and seep symbionts has only just begun to be studied. Fine-scale variations in isotopic composition and correlative physiological parameters may help to define optimal environments for each species.

Collection of vent and seep biota for isotopic analyses in many instances has been relatively non-systemtatic. Despite this opportunistic approach, $\delta^{13}C$ and $\delta^{15}N$ values of symbiont species have provided significant insight into the novelty of chemosynthetic symbioses of tubeworms and bivalves and raised many questions. Isotopic compositions of symbiont species are now used as powerful tools to target organisms of particular interest to biochemists and physiologists. Sampling strategies focused on animals collected along gradients of water chemistry and in conjunction with fine scale sampling and isotopic analyses of nutrient parameters can be

developed to identify the most likely sources of carbon and nitrogen for symbiont species. As patterns in isotopic compositions of field populations are resolved, laboratory studies involving isotopic analyses will become critical for our understanding of interactions among hosts, endosymbionts, and nutrient sources, and the expression of these interactions as isotopic compositions.

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Table 1. Carbon and nitrogen isotopic compositions of vent and seep symbiont tissues. Bold figures are plotted in Figure 2. RG = Rose Garden, Galapagos Spreading Center; GE = Garden Eden, Galapagos Spreading Center; CB = Clambake, Galapagos Spreading Center; CA = Clam Acres, 21°N East Pacific Rise HG = Hanging Gardens, 21°N East Pacific Rise; EPR = 12°N, East Pacific Rise; MA = Marianas Back Arc Basin; FL = Florida Escarpment; LA = Louisiana seeps; OR = Oregon Subduction Zone; NC = Northern California seeps; JT = Japan Trench; SB = Sagami Bay; LF = Laurentian Fan.

Species	Locale	Carbon	Nitrogen	References
VENT VESTIMENTIFERA				
Rifita pachyptila				
vestimentum	RG	-10.9 (2?)	+3.8 to + 4.0	Rau 1981a,b
"tissue"	GE	-10.9 (1)	+3.9 (1)	Williams et al. 1981
vestimentum	RG	-11.2 (2)	+4.5 (2)	Fisher et al. 1988a
vestimentum	HG	-11.7 (1)	+4.5 (1)	Van Dover & Fry Submitted
average:		-11.2	+3.7	
trophosome	RG	-11.0 (2?)	+1.8 to +2.0	Rau 1981a,b
trophosome	RG	-10.9±0.2 (25)	+1.8±0.2 (13)	Fisher et al. 1988a
trophosome	HG	-11.3 (1)	+3.4 (1)	Van Dover & Fry Submitted
Oasisia alvinae				
vestimentum	HG	-11.4 (1)	+2.9 (1)	Van Dover & Fry Submitted
trophosome	HG	-10.4 (1)	+3.4 (1)	Van Dover & Fry Submitted
SEEP VESTIMENTIFERA				
Escarpia laminata				
"tissue"	FL	-42.7±0.7 (3?)	-2.8 (2?)	Paull et al. 1985
vestimentum	FL	-44.8±3.5 (3)		Cary et al. Submitted
trophosome	FL	-47.2±2.4 (4)		Cary et al. Submitted
Escarpia-like sp.				
"tubes and tissues"	LA	-21.4 to -48.6 (24)		Brooks et al. 1987
"tissues"	LA	-40.9 to -30.4 (3)	+4.1 (2)	Brooks et al. 1987
Lamellibrachia sp.				
"flesh"	LA	-27.0 (1)		Kennicutt et al. 1985
"tubes and tissues"	LA	-29.8 to -57.2 (37)		Brooks et al. 1987
"tissue"	LA	-43.2 (1)	+2.7 (1)	Brooks et al. 1987
Lamellibrachia barhami				
"tissue"	OR	-31.9 ("composite")		Kulm et al. 1986
Lamellibrachia sp.				
trophosome	SB	-25.8 (1)	-4.1 (1)	Saino & Ohta In Press

Table 1. Continued.

VENT BIVALVES					
Calyptogena magnifica					
mantle	RG	-32.0	(2?)	+2.4 (2?)	Rau 1981a,b; 1985
mantle	CA	-32.6	(2?)	+4.0 (2?)	Rau 1981a,b; 1985
"rest"	RG	-32.6±0.5	(27)	+4.5±1.3 (10)	Fisher et al. 1988c
gill	RG	-33.2±0.7	(27)	+1.8±1.3 (10)	Fisher et al. 1988c
Bathymodiolus thermophilus					
"tissue"	MB	-33.5	(3)		Williams et al. 1981
mantle and foot	CB	-33.2	(1)		Rau & Hedges 1981
foot	RG	-32.9	(1)	+1.7 (1)	Van Dover & Fry Submitted
"rest"	RG				
central population		-34.4±0.8	(28)		Fisher et al. 1988b
peripheral population		-33.5±0.8	(11)		Fisher et al. 1988b
"gill"	RG				
central population		-35.7±0.7	(28)		Fisher et al. 1988b
peripheral population		-34.7±0.9	(11)		Fisher et al. 1988b
"tissue"	RG				
central population				-3.9±0.6 (6)	Fisher et al. 1988b
peripheral population				+3.5±0.7 (8)	Fisher et al. 1988b
Bathymodiolus sp.					
mantle	MA	-32.8	(1)	-0.5 (1)	Van Dover & Fry Submitted
gill	MA	-34.8	(1)	-3.0 (1)	Van Dover & Fry Submitted
SEEP BIVALVES					
Calyptogena pacifica					
"body"	NC	-36.3	(2)		Kennicutt et al. In Press
gill	NC	-36.2	(2)		Kennicutt et al. In Press
Calyptogena ponderosa					
"soft tissue"	LA	-35.4	(2)		Kennicutt et al. 1985
"tissue"	LA	-36.9 to -39.1	(3)		Brooks et al. 1987
"soft tissue"	LA	-34.9	(2)	+1.1 to +7.1 (3)	Brooks et al. 1987
average		-36.6		+4.1	
Calyptogena phaseoliformis					
mantle	JT	-38.1	(1)	+3.3 (1)	Boulegue et al. 1987
mantle	JT	-36.7	(1)	-0.8 (1)	Saino & Ohta In Press
average		-37.4		+1.2	
gill	JT	-40.1	(1)	-2.8 (1)	Boulegue et al. 1987
gill	JT	-38.7	(1)	-3.3 (1)	Saino & Ohta In Press

Table 1. Continued.

Calyptogena soyae						
mantle	SB	-35.0	(1)	-6.1	(1)	Saino & Ohta In Press
gill	SB	-35.3	(1)	-9.6	(1)	Saino & Ohta In Press
Calyptogena sp.						
gill	OR	-51.6	(1)			Kulm et al. 1986
Calyptogena sp.						
tissue	FL	-36.7	(3)			Cary et al. Submitted
Calyptogena sp.						
foot, mantle	LF	-34.7	(1)	-1.3	(1)	Van Dover and Fry, Unpublished
Vesicomya cordata						
"soft tissue"	OR	-36.3	(1)	-0.9	(1)	Kulm et al. 1987
Bathymodiolus sp.						
tissue	FL	-74.3±2.0	(10?)	-8.9	(2?)	Paull et al. 1985
mantle	FL	-73.0±2.2	(5)			Cary et al. Submitted
gill	FL	-72.9±1.1	(5)			Cary et al. Submitted
Bathymodiolus sp.						
??	LA	-40.1 to -57.6	(38)			Brooks et al. 1987
soft tissue	LA	-45.5 to -50.1	(9)	-12.9 to +3.0	(10)	Brooks et al. 1987
mid-point				-5.0		
mantle	LA	-53.9±2.9	(3)			Childress et al. 1986
gill	LA	-51.8±0.2	(3)			Childress et al. 1986

FIGURE LEGENDS

Figure 1. Map showing the locations of vents (o) and seeps (•) discussed in this paper.

Figure 2. $\delta^{13}C_{PDB}$ vs. $\delta^{15}N_{AIR}$ for vent and seep symbionts.

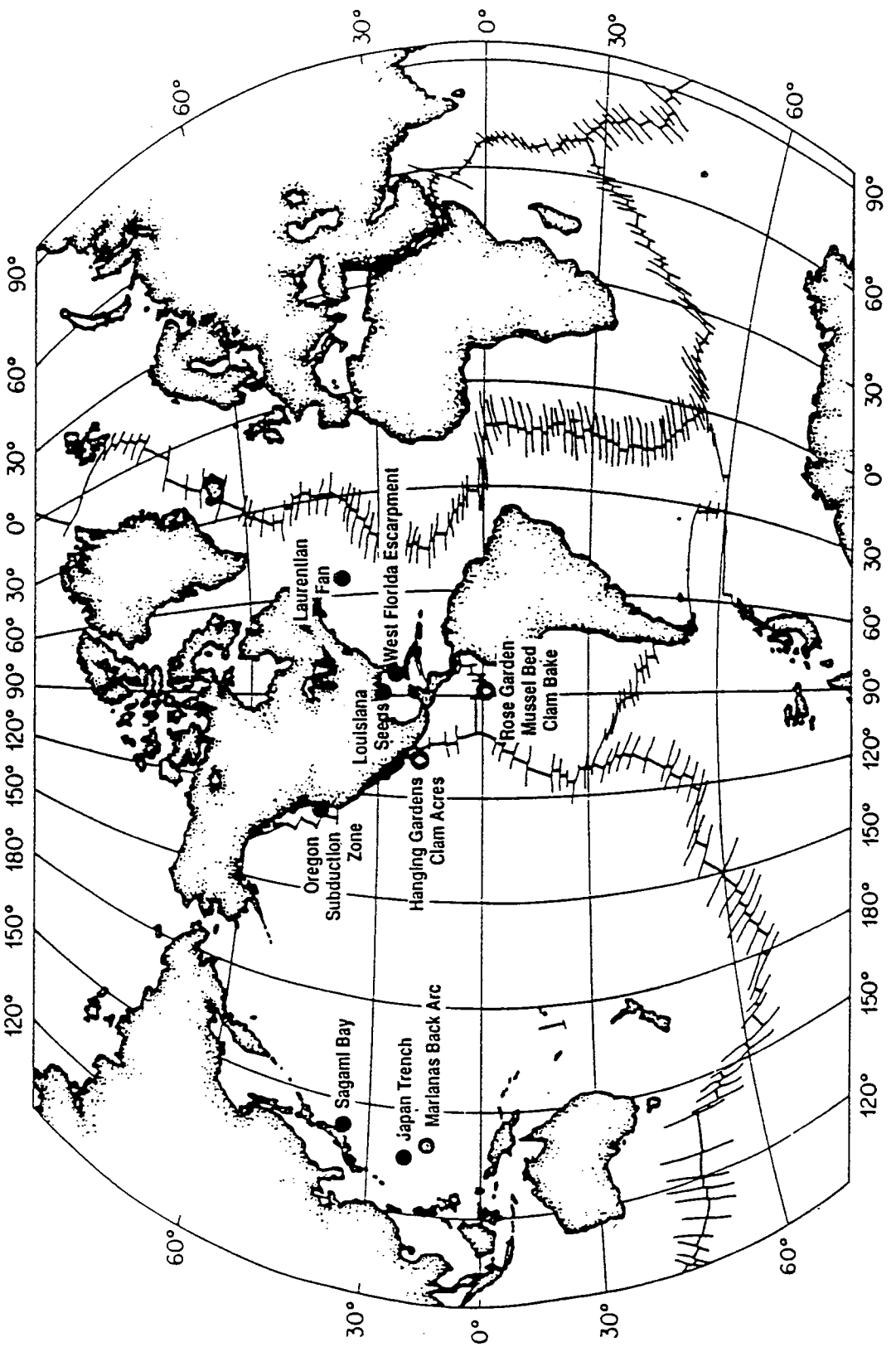
Laurentian Fan
West Florida Escarpment
Louisiana Seeps
Rose Garden
Mussel Bed
Clam Bake
Oregon Subduction Zone
Hanging Gardens
Clam Acres
Sagami Bay
Japan Trench
Marianas Back Arc

Figure 1

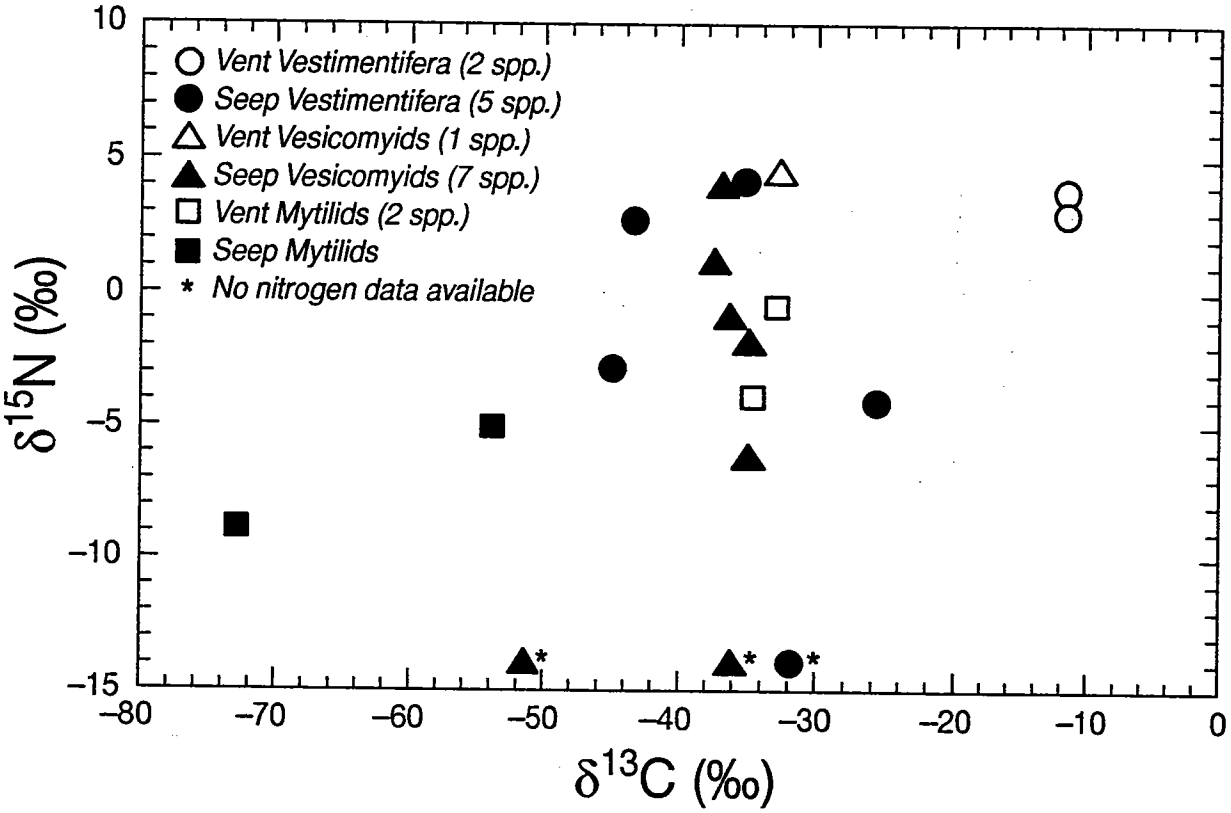
Vent Vestimentifera (2 spp.)
Seep Vestimentifera (5 spp.)
Vent Vesicomyids (1 spp.)
Seep Vesicomyids (7 spp.)
Vent Mytilids (2 spp.)
Seep Mytilids
* No nitrogen data available
10
5
0
−5
−10
−15
−80
−70
−60
−50
−40
−30
−20
−10
0
δ15N (‰)
δ13C (‰)

Figure 2

Chapter 6

Patterns in isotopic compositions of deep-sea vent and seep animal-symbiont associations

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ABSTRACT

We present new data on $\delta^{13}C$ and $\delta^{15}N$ compositions of vent and seep animal-symbiont associations from the East Pacific Rise (11°N), Guaymas Basin, Gorda Ridge, Juan de Fuca Ridge, Florida Escarpment and Laurentian Fan. Using a compilation of this data and published values, we have identified the following patterns in the isotopic compositions of animal-symbiont associations:

* All vent and seep invertebrate species with bacterial endosymbionts, i.e. vestimentiferan tubeworms and vent bivalves (vesicomyid clams and mytilid mussels) have $\delta^{13}C$ values that lie outside the range of values associated with photosynthetic processes and outside the range of values measured in non-vent deep-sea fauna.
* Vent tubeworms have $\delta^{13}C$ values centered around -13°/oo and are consistently and significantly enriched in ^{13}C relative to vent bivalves.
* Seep tubeworms have lighter and more variable $\delta^{13}C$ values than vent tubeworms.
* $\delta^{15}N$ values of vent and seep tubeworms and bivalves species are variable but consistently lighter than deep-water sources of particulate organic nitrogen.

Distinctive carbon isotopic compositions of non-photosynthetically-derived organic material indicate that $\delta^{13}C$ has potential as a tracer of chemosynthetic production into the surrounding pelagic and benthic environments. The consistency of $\delta^{13}C$ values among diverse tubeworm and bivalve species at vents is attributed to a large, isotopically well-buffered source pool of inorganic carbon, namely deep-water DIC.

Variability in seep tubeworm $\delta^{13}C$ values may be attributed to significant contributions of seep effluent DIC with locally variable $\delta^{13}C$ values. $\delta^{15}N$ values of vent and seep animal-symbiont associations implicate endosymbiotic bacteria in the assimilation of local, inorganic nitrogen sources. The broad range of $\delta^{15}N$ values in vent and seep tubeworms and bivalves suggests that sources of nitrogen are not well-buffered and are highly site and microhabitat specific.

INTRODUCTION

Chemoautotrophic production based on microbial oxidation of reduced sulfur compounds and methane supports communities of invertebrates at deep-sea hydrothermal vents and cold seeps. Endosymbiotic relationships of autotrophic bacteria within invertebrate tissues are characteristic of most species (herein called symbiont species) that dominate the biomass of vent and seep communities. Translocation of bacterially-fixed organic carbon is presumed to provide a large proportion of the nutritional requirements of host invertebrates.

Carbon and nitrogen isotopic compositions of host tissues have been used to demonstrate the dependence of vent and seep symbiont species on chemosynthetically-derived organic material (reviewed in Rau 1985; Karl 1987; Southward 1987; Van Dover 1989). In this paper, we report new data on isotopic compositions of symbionts collected from vent and seep environments. We then examine and interpret patterns of carbon and nitrogen isotopic compositions of representative symbiont species from chemosynthetic communities.

METHODS

Species were collected in 1988 during *Alvin* dives at vent sites on the East Pacific Rise near 11°N, Guaymas Basin (Southern Trough), Gorda Ridge (Northern Escanaba Trough) and Juan de Fuca Ridge (Endeavour Segment). Seep symbionts were collected during an *Alvin* Dive at the Florida Escarpment and by Day Dredge at the Laurentian Fan. Collection data are provided in Table 1; locations of vent and seep sites are shown in Figure 1.

All specimens were stored frozen until prepared for isotopic analysis. Frozen tissues were thawed and acidified with 0.1 N HCl to remove contaminating carbonates, dried at 60°C, and analyzed for carbon and nitrogen stable isotopic compositions following the methods of Minagawa et al. (1984). CO_2 and N_2 gases were analyzed separately with a Finnigan MAT 251 isotope-ratio mass spectrometer.

Isotopic compositions are expressed in terms of °/oo differences from a standard, where:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3, \text{ and}$$

$$X = {}^{13}C \text{ or } {}^{15}N$$

$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N.$$

Standard reference materials are carbon in the Pee Dee Belemnite (PDB) or nitrogen gas in the atmosphere (AIR). "Lighter" or "more negative" δ values are enriched in the lighter isotope and reciprocally depleted in the heavier isotope.

RESULTS AND DISCUSSION

A compilation of carbon and nitrogen data for vent and seep symbionts is provided in Table 2 and plotted in Figure 2. Average values for populations are given where possible. Our discussion (and Figures 2 and 3) focuses on isotopic compositions of invertebrate host tissues that lack symbiotic bacteria (i.e., non-gill tissues in bivalves; non-trophosome tissues in tubeworms). Species identifications in three instances are uncertain (Guaymas Basin *Riftia*, Gorda Ridge *Ridgea*, EPR (11°N) *Bathymodiolus*); we have chosen to treat these geographically distinct populations as separate species. Included in our compilation are 8 species of vent tubeworms

(Vestimentifera), 5 species of seep tubeworms, 3 species of vent clams (Vesicomyidae), 8 species of seep clams, 3 species of vent mussels (Mytilidae), and 2 species of seep mussels. Symbiotic associations in some species have not been demonstrated directly but are inferred from analogy with congeneric or confamilial species. Table 3 outlines the available non-isotopic evidence for either methane- or sulfur-based metabolism in vent and seep symbionts.

DETERMINANTS OF $\delta^{13}C$ COMPOSITION OF CHEMOSYNTHETICALLY FIXED CO_2

Two important determinants of the carbon isotopic composition of organic tissues are i) the $\delta^{13}C$ composition of the primary carbon source assimilated *via* CO_2 fixation or during oxidation of C_1 compounds and ii) biochemical fractionation effects during assimilation of these sources. Before attempting to interpret the carbon isotopic data, we identify important carbon sources available to vent and seep symbiont species and briefly review potential biochemical fractionation effects.

Primary Carbon Sources

Dissolved Inorganic Carbon. Sulfide-dependent chemosynthetic symbioses fix dissolved inorganic carbon (DIC) *via* autotrophic biochemical pathways. Several isotopic pools of DIC can be identified:

* In the deep ocean, DIC is a large pool with a fairly uniform $\delta^{13}C$ of ~0 °/oo (Kroopnick 1985)
* Small, local variations in $\delta^{13}C$ of DIC are associated with CO_2 enrichment at active vents and are of geochemical origin. These variations are not well-documented, but at

two low-temperature vent sites on the Galapagos Spreading Center, ΣCO_2 = 9280 μM/kg and $\delta^{13}C$ ranged from -5.1 to -5.9 °/oo in end-member (350°C) fluids (Craig et al. 1980).

* Larger variations in $\delta^{13}C$ of DIC may occur within porewaters of sedimented, hydrocarbon-rich vents. Martens et al. (pers. comm.) report gradients in $\delta^{13}C$ values of porewater DIC with depth ($\delta^{13}C$ = -17 °/oo and $[CO_2]$ = 17 mM at 20 cm) in Guaymas Basin hydrothermal sediments; ^{13}C-depletion in porewater CO_2 is attributed to diagenesis of surface-derived particulate organic material. Large, localized variations in $\delta^{13}C$ values of DIC are also associated with seep effluents ($\delta^{13}C$ = ~-10 to -15 °/oo; e.g. Brooks et al. 1984) and are attributed to biological respiration of isotopically light methane (Brooks et al. 1987).

Respired CO_2 in host tissues may form an additional pool of isotopically light CO_2 available to endosymbiotic bacteria. But because respiration involves little fractionation of CO_2, bacterial use of respired CO_2 may not be detectable.

Methane. Methane-dependent chemosynthetic symbioses oxidize methane to produce organic carbon compounds. Three isotopically distinct pools of methane may be available to vent and/or seep organisms:

* Biogenic methane of microbial origin under reducing conditions has isotopically light $\delta^{13}C$ values of -60 to -90 °/oo (Claypool and Kaplan 1974).

High activities of RuBPCase in some vent and seep symbiont species indicate that autotrophic fixation of DIC occurs in endosymbiotic sulfide-oxidizing autotrophic bacteria. Ruby et al. (1987) found a 17 °/oo fractionation vs. free CO_2 toward lighter $\delta^{13}C$ values in two laboratory-isolated species of free-living, sulfide-oxidizing chemoautotrophic bacteria (i.e., $\delta^{13}C_{CO2} - \delta^{13}C_{bacteria} = 17$ °/oo). This fractionation factor is consistent with the generalization that CO_2 fixation in autotrophic bacteria follows the same biochemical pathway as CO_2 fixation in C_3 plants.

Fractionation Effects during Assimilation of Methane

Methane serves as a primary carbon source for methane-oxidizing bacteria. Zyakun et al. (1981) report a 10 to 20 °/oo isotopic fractionation in methane-oxidizing bacteria (i.e. $\delta^{13}C_{methane} - \delta^{13}C_{bacteria} = 10\text{-}20$ °/oo).

Isotopic variations introduced at the level of CO_2 fixation or methane assimilation are typically passed on through metabolic and trophic transformations with little or no alteration (reviewed in Fry and Sherr 1984, Peterson and Fry 1987), i.e. consumer tissues reflect the average $\delta^{13}C$ ratio of their diet. We can thus expect $\delta^{13}C$ values of bacterial and host tissues of symbiotic associations to be similar.

Based on the above discussion, we can identify expected ranges of $\delta^{13}C$ values for different metabolic pathways of carbon incorporation. These are plotted in Figure 3, together with ranges of $\delta^{13}C$ values observed in vent and seep symbiont species. Overlapping ranges in expected and observed values emphasize the potential for ambiguity in

* Thermogenic methane has a carbon isotopic composition heavier than -55 °/oo (Frank et al. 1974). High concentrations of methane (14000 μM) with $\delta^{13}C$ values of -40 to -50 °/oo have been measured at Guaymas basin vents and are presumed to be of thermocatalytic origin (Welhan and Craig 1982; Chris Martens, pers. comm.). Thermogenic methane has also been detected at Louisiana slope hydrocarbon seeps ($\delta^{13}C$ >-45 °/oo; Brooks et al. 1987) and Northern California slope hydrocarbon seeps ($\delta^{13}C$ = -43 to -44 °/oo; Field and Kvenvolden 1985).
* Crustal methane emitted from high temperature vents has a measured $\delta^{13}C$ value of -15 to -17 °/oo. Concentrations of crustal methane vary among vent sites. Significant concentrations, as high as 1300 μM, have been measured at Juan de Fuca vents (M. Lilley, Pers. Comm.). At 21°N vents on the East Pacific Rise, methane concentrations of 65 μM in vent water are 10^5 times normal deep-ocean concentrations (Lilley et al. 1983, Welhan and Craig 1983).

Fractionation Effects during Fixation of CO_2

The difference in fractionation effects in open vs. closed autotrophic pathways is important in our interpretation of carbon isotopic composition of symbiont species. In open systems, where CO_2 is unlimited, both equilibrium and chemical fractionation effects can be expressed. In closed systems, where CO_2 is limiting, only the equilibrium fractionation effect is expressed.

According to theoretical models (O'Leary 1988), regardless of metabolic pathway, there is an ~8 °/oo fractionation associated with the equilibrium exchange between DIC and $CO_{2(aq)}$ prior to fixation (Emrich et al. 1970). Where CO_2 is unlimiting, there is an additional 29 °/oo fractionation associated with the enzymatic fixation of $CO_{2(aq)}$ by ribulose-bisphosphate carboxylase (RuBPCase; Roeske and O'Leary 1984). These figures yield a total, maximum fractionation of 39 °/oo for autotrophically-fixed carbon. This 39 °/oo fractionation is superimposed on the isotopic composition of the source carbon. In plants, this fractionation scheme is most closely followed in species that have a C_3 pathway for CO_2-fixation.

In closed autotrophic pathways, under conditions where CO_2 is limiting, all of the $CO_{2(aq)}$ that reaches the RuBPCase-catalyzed step is fixed; i.e., there can be no discrimination by the enzyme RuBPCase and no 29 o/oo fractionation effect at this step. Thus, where CO_2 is limiting, only the 8 °/oo equilibrium fractionation is expected (reviewed in O'Leary 1988). Again, this 8 °/oo fractionation is superimposed on the isotopic composition of the source carbon. This fractionation scheme is most closely followed in plants that have a C_4 CO_2-fixation pathway which involves the sequential operation of two carboxylase enzyme systems. The first system, phosphoenolpyruvate carboxylase (PEPCase), is the irreversible step. A small, 2 °/oo fractionation is associated with this step. The malate or aspartate product of this step is transported, decarboxylated and refixed by RuBPCase. Because the PEPCase reaction is irreversible, all of the CO_2 that reaches the RuBPCase step is fixed and there can be no further fractionation effect.

tubeworms and bivalves argues for sulfur-based, chemoautotrophic metabolism in all vent symbiont species within the known data base of Table 2.

The dichotomy in the carbon isotopic compositions of vent tubeworms and bivalves could be a consequence of differences in the isotopic composition of the source carbon and/or differences in biochemical fractionation effects. Several lines of evidence suggest that source differences are not responsible for the 20 °/oo difference in vent tubeworms and bivalves:

* Both taxa occur in similar microhabitats.
* Radiocarbon analyses indicate that they use the same DIC source (Williams et al. 1981).
* The consistency of $\delta^{13}C$ values within diverse vent tubeworm populations and within diverse bivalve populations is further evidence for a large, isotopically well-buffered source pool of inorganic carbon, namely deep-water DIC with small, 1-2 °/oo variations introduced by contributions of magmatic DIC or from use of dissolved and particulate organic carbon.

Spiro et al. (1986) suggest that the tubeworm-bivalve carbon isotopic dichotomy might be temperature-related. Their premise is two-fold: i) high temperatures reduce selectivity of the carboxylase enzyme (Sackett et al. 1965; Galimov 1985) and ii) vent tubeworms and bivalves occupy different thermal environments. The relationship between $\delta^{13}C$ and temperature was based on field correlations between $\delta^{13}C$ values of surface plankton and surface seawater temperatures. Subsequent work in the field and laboratory have shown that factors other than temperature contributed to the observed field variations

interpretation of isotopic compositions in the absence of complementary studies on biochemical and physiological attributes of individual species.

PATTERNS IN $\delta^{13}C$ COMPOSITION OF VENT AND SEEP SYMBIONTS

Vent and seep symbionts have carbon isotopic compositions that lie outside the range of values associated with surface-derived photosynthetic processes (-15 to -25 °/oo; Rau 1985) and outside the range of values associated with non-vent deep-sea fauna (-17 to -21 °/oo; Van Dover and Fry In Press). This holds true regardless of variation in parameters such as depth (ranging from 450 m at the N. California seep site to nearly 6000 m in the Japan trench), geochemistry (sulfide-rich vent waters, hydrocarbon-rich vents, hydrocarbon-rich seeps, methane-rich seeps), source of reduced energy (H_2S or CH_4), metabolic pathway, geographic location (eastern Pacific, Gulf of Mexico, Oregon Subduction Zone, Marianas Back Arc Basin), and taxonomy (bivalves, tubeworms). Carbon isotopic composition thus has potential as a tracer for transfer of non-photosynthetic production into surrounding pelagic and benthic environments.

Vent tubeworms have $\delta^{13}C$ values centered around -13 °/oo ($x = -12.7 \pm 2.2$ °/oo S.D.; $n = 8$ species) and are consistently and conspicuously about 20 °/oo heavier than vent bivalves (-32 to -36 °/oo). This pattern prevails across diverse vent sites. While non-isotopic evidence for sulfur-based metabolism is available for only 3 vent symbiont species (*Riftia pachyptila, Calyptogena magnifica, Bathymodiolus thermophilus*; see Table 3), similarities in carbon isotopic compositions of these known sulfide-oxidizers and other vent

(Degens et al. 1968; Rau et al. 1982). Laboratory experiments with C_3 and C_4 plants showed little (3 °/oo) or no shift in $\delta^{13}C$ compositions of tissues with a 15°C difference in growth temperature (O'Leary and Treichel, unpublished; cited in O'Leary 1988). Given such a small temperature effect in plants and the limited range of temperatures over which bivalves and tubeworms co-occur (~0-10°C), it is difficult to attribute a significant portion of the 20 °/oo difference in $\delta^{13}C$ values of vent bivalves and tubeworms to temperature.

Source and/or temperature effects seem unlikely to provide an acceptable explanation for the tubeworm/bivalve dichotomy in carbon isotopic composition. An alternative explanation is that there is a basic metabolic difference in the CO_2 fixation pathway between tubeworms and bivalves. Rau (1981a) noted the resemblance of $\delta^{13}C$ values of tubeworms to isotopic compositions of C_4 plants (CO_2-limited; Figure 3) while bivalves have highly fractionated $\delta^{13}C$ values vs CO_2 (CO_2-unlimited; Figure 3). Subsequent biochemical studies by Felbeck (1981, 1985), Felbeck and Somero (1982) support the hypothesis that tubeworms have a two-step CO_2 fixation pathway analogous to that found in C_4 plants, although activity of PEPCase remains undetected.

Vent tubeworm $\delta^{13}C$ values also resemble $\delta^{13}C$ values inferred for free-living microorganisms consumed by heterotrophic components (shrimp, polychaetes, amphipods, etc.) of vent food webs (Van Dover et al. 1988; Van Dover and Fry, In Press).

The dichotomy in carbon isotopic composition of tubeworms and bivalves is not maintained at seeps (Figures 2 and 3). Seep tubeworms are isotopically lighter than vent tubeworms and are isotopically more

variable than vent tubeworms, vent bivalves (mytilids or vesicomyids), or seep vesicomyid bivalves. With a single exception (Oregon Seep *Calyptogena* sp.), $\delta^{13}C$ values of seep vesicomyids (7 species, 6 locations) are constrained within a 4 °/oo range around an average of -36 °/oo (2 °/oo lighter than vent vesicomyids). Two vesicomyid species are known from both vents and seeps: *Calyptogena phaseoliformis* is found at Gorda Ridge vents ($\delta^{13}C$ = -35.9 °/oo) and at Japan Trench seeps ($\delta^{13}C$ = -37.4 o/oo); *Vesicomya gigas* occurs at Guaymas basin vents ($\delta^{13}C$ = - 35.8 °/oo) and Northern California seeps ($\delta^{13}C$ = - 36.3 °/oo). Non-isotopic evidence for sulfur-based metabolism in seep bivalves is available for 4 species (*Calyptogena ponderosa, C. phaseoliformis, Vesicomya gigas, V. cordata*; see Table 3). These data suggest the hypothesis that seep and vent vesicomyids with $\delta^{13}C$ values between -30 and -40 °/oo have a sulfur-based metabolism; the uniformity among $\delta^{13}C$ values argues again for reliance on a large, isotopically well-buffered inorganic carbon source, namely bottom water DIC. Oregon seep *Calyptogena* sp., with $\delta^{13}C$ values of -51.6 (gills), stand as an exception. Two explanations for this deviation from the normal pattern observed in vesicomyids can be hypothesized: *a)* metabolism in the Oregon species is sulfur-based, but the clam has access to a local, ^{13}C-depleted inorganic carbon source or *b)* metabolism in this species is not based on sulfur oxidation. Kulm et al. (1986) argue for methane oxidation in Oregon seep *Calyptogena* sp.

$\delta^{13}C$ values of seep vestimentiferans are variable both among species (-25.8 to -44.8 °/oo) and within populations of a given species (e.g., a range of -30.4 to -40.9 °/oo in the Louisiana

Escarpia-like vestimentiferan). This variability is particularly interesting in the case of two closely-related species in the family Escarpiidae from Florida and Louisiana seeps (Table 2) given the evidence for sulfide-based metabolism in both species (Table 3). If variability in seep tubeworm $\delta^{13}C$ values is attributed to differences in local $\delta^{13}C$ values of DIC between sites, one then has to account for the similarity in $\delta^{13}C$ values of sulfide-based symbioses in *Calyptogena* species (FL: -36.9, LA: -36.6 °/oo) from the same locations. One hypothesis that satisfies this constraint is that the plumes (obturacula) of seep tubeworms, where absorption of HS^- and DIC takes place (Felbeck and Somero 1982), must be positioned where the flux of chemically-altered seawater is greatest; $\delta^{13}C$ values of tubeworm tissues can thus reflect very localized differences in $\delta^{13}C$ values of DIC associated with this flux. In contrast, seep bivalves may need only position themselves so that their foot, where absorption of HS^- has been suggested to take place (Arp et al. 1984), occupies a sulfide-rich microhabitat while the gills, where DIC uptake occurs, can draw in isotopically uniform ambient seawater. Ten to 15 °/oo variations in local DIC $\delta^{13}C$ values at seeps can be expected from diagenetic processes such as bacterial degradation of seeping oil, gas and surface-derived organic material (Brooks et al. 1984).

Rau (1985) suggested that the difference in $\delta^{13}C$ values of seep and vent tubeworms was related to size, with smaller species characteristic of seeps, such as *Lamellibrachia* sp., experiencing less of a carbon limitation than the larger vent species, *Riftia pachyptila*. Data reported here on isotopic compositions of other, small vestimentiferan species that occupy vent habitats (*Oasisia*

alvinae, Ridgea sp., Tevnia jerichonana; $\delta^{13}C$ = -10.7 to -12.7 °/oo) refute this hypothesis.

DETERMINANTS OF $\delta^{15}N$ *COMPOSITION OF VENT AND SEEP SYMBIONTS*

Nitrogen isotopic composition of organic material is a function of the isotopic composition of the inorganic precursor (N_2, NO_3^-, NH_4^+) and fractionation during fixation or assimilation.

Miyake and Wada (1967) have measured the isotopic composition of inorganic nitrogen in the western North Pacific Ocean: In deep water, (> 1000 m), nitrate is the most abundant form of nitrogen (~35 μM), with a $\delta^{15}N$ value ranging from 5.1 to 7.5 °/oo. Fractionation during assimilation of NO_3^- is about 15 °/oo. Dissolved N_2 at depths > 1000m has $\delta^{15}N$ values ranging from 0.4 to 1.7 °/oo; fractionation during N_2-fixation is about 1 °/oo. Ammonia concentrations at depth are low, on the order of 0.4 μg atoms/l; at 500 m, $\delta^{15}N$ (NH_3) is 6.5 to 7.5 °/oo. Localized enrichments in NH_3 concentration are reported for vent and seep effluents ($[NH_4^+] < 5$ μM; Johnson et al. 1988), but $\delta^{15}N$ values for this pool of inorganic nitrogen have not been measured. Where ammonia is present, it is likely to be an important species in nitrogen assimilation; fractionation during assimilation of NH_3^+ may be large (up to ~ 21 °/oo; M. Fogel, Pers. Comm.). Figure 4 summarizes possible fractionation effects during nitrogen assimilation and emphasizes the overlap in $\delta^{15}N$ values of resulting organic material and consequent ambiguity in interpretation of nitrogen sources based solely on nitrogen isotopic composition of organic tissues.

$\delta^{15}N$ values of vent and seep symbionts are consistently lighter than deep-water sources of particulate organic nitrogen, implicating endosymbiotic bacteria in the assimilation of local, inorganic nitrogen. The degree of overlap in nitrogen isotopic fractionation effects during assimilation of inorganic nitrogen sources (Figure 4) prevents us from determining the relative importance of these sources in symbiont species. The broad range of nitrogen isotopic compositions of vent and seep symbionts suggests that sources of nitrogen are not well-buffered and are highly site and microhabitat specific.

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Table 1. Collection data for vent and seep symbiont species.

	Latitude; Longitude	Alvin Dive Number	Depth (m)	General Description of Site
EAST PACIFIC RISE				
Tevnia jerichonana	10°56'N	1986	2558	Unpublished
Bathymodiolus sp. 1	11°25'N	1993	2510	"
GUAYMAS BASIN				
Riftia sp.	27°00'N	1613	2000	Grassle 1986
Vesicomya gigas	"	1616	"	"
GORDA RIDGE				
Ridgea sp. 1	41°00'N	2036	3239	Zierenberg et al. 1988; Campbell et al. 1988 Van Dover et al. In Press
Calyptogena phaseoliformis	"	2042	"	"
JUAN de FUCA RIDGE, Endeavour Segment				
Ridgea sp. 2	47°57'N	2056	2250	Tivey and Delaney 1986
Ridgea sp. 3	"	2061	"	"
Ridgea sp. 4	"	2061	"	"
FLORIDA ESCARPMENT				
Calyptogena sp. 1	26°02'N; 84°55'W	1754	3270	Paull et al. 1984; Hecker 1985
LAURENTIAN FAN				
Calyptogena sp. 2	43°30'N; 55°30'W	---	3800	Mayer et al. 1988

Table 2. Carbon and nitrogen isotopic compositions of vent and seep symbiont tissues. Bold figures are plotted in Figure 3. Numbers in parentheses correspond to number of analyses. RG = Rose Garden, Galapagos Spreading Center; GE = Garden Eden, Galapagos Spreading Center; CB = Clambake, Galapagos Spreading Center; CA = Clam Acres, 21°N East Pacific Rise; HG = Hanging Gardens, 21°N East Pacific Rise; GR = Gorda Ridge; EPR = 12°N, East Pacific Rise; GB = Guaymas Basin; MA = Marianas Back Arc Basin; FL = Florida Escarpment; LA = Louisiana Seeps; OR = Oregon Subduction Zone; NC = Northern California seeps; JT = Japan Trench; SB = Sagami Bay; LF = Laurentian Fan.

Species	Locale	Carbon		Nitrogen		References
VENT VESTIMENTIFERA						
Rifita pachyptila						
vestimentum	RG	-10.9	(2?	+3.8 to + 4.0		Rau 1981a,b
"tissue"	GE	-10.9	(1)	+3.9	(1)	Williams et al. 1981
vestimentum	RG	-11.2	(2)	+4.5	(2)	Fisher et al. 1988a
vestimentum	HG	-11.7	(1)	+4.5	(1)	Van Dover & Fry In Press
average:		**-11.2**		**+3.7**		
trophosome	RG	-11.0	(2?)	+1.8 to +2.0		Rau 1981a,b
trophosome	RG	-10.9±0.2	(25)	+1.8±0.2	(13)	Fisher et al. 1988a
trophosome	HG	-11.3	(1)	+3.4	(1)	Van Dover & Fry In Press
Riftia sp.						
vestimentum	**GB**	**-14.2**	**(1)**	**+1.0**	**(1)**	**this report**
trophosome	GB	-13.9	(1)	+1.0	(1)	this report
Oasisia alvinae						
vestimentum	**HG**	**-11.4**	**(1)**	**+2.9**	**(1)**	**Van Dover & Fry In Press**
trophosome	HG	-10.4	(1)	+3.4	(1)	Van Dover & Fry In Press
Ridgea sp. 1						
vestimentum	**GR**	**-12.7**	**(1)**	**+1.2**	**(1)**	**this report**
trophosome	GR	-13.6	(1)	-1.5	(1)	this report
Ridgea sp. 2						
vestimentum	**JdeF**	**-16.4**	**(1)**	**+2.4**	**(1)**	**this report**
trophosome	JdeF	-17.1	(1)	+2.2	(1)	this report
Ridgea sp. 3						
vestimentum	**JdeF**	**-10.5**	**(1)**	**+0.8**	**(1)**	**this report**
trophosome	JdeF	-11.0	(1)	+0.9	(1)	this report
Ridgea sp. 4						
vestimentum (4 pooled)	**JdeF**	**-14.8**	**(1)**	**+2.2**	**(1)**	**this report**
trophosome (4 pooled)	JdeF	-15.8	(1)	+1.6	(1)	this report
Tevnia jerichonana						
vestimentum	**EPR**	**-10.7**	**(1)**	**+4.0**	**(1)**	**this report**
trophosome	EPR	-8.4	(1)	+2.9	(1)	this report

Table 2. Continued.

SEEP VESTIMENTIFERA					
Escarpia laminata					
"tissue"	FL	-42.7±0.7 (3?)	-2.8	(2?)	Paull et al. 1985
vestimentum	**FL**	**-44.8±3.5 (3)**			**Cary et al. Submitted**
trophosome	FL	-47.2±2.4 (4)			Cary et al. Submitted
Escarpia-like sp.					
"tubes and tissues"	LA	-21.4 to -48.6 (24)			Brooks et al. 1987
"tissues"	**LA**	**-40.9 to -30.4 (3)**	**+4.1**	**(2)**	**Brooks et al. 1987**
Lamellibrachia sp.					
"flesh"	**LA**	**-27.0 (1)**			**Kennicutt et al. 1985**
"tubes and tissues"	LA	-29.8 to -57.2 (37)			Brooks et al. 1987
"tissue"	**LA**	**-43.2 (1)**	**+2.7**	**(1)**	**Brooks et al. 1987**
Lamellibrachia barhami					
"tissue"	**OR**	**-31.9 ("composite")**			**Kulm et al. 1986**
Lamellibrachia sp.					
trophosome	**SB**	**-25.8 (1)**	**-4.1**	**(1)**	**Saino & Ohta In Press**
VENT BIVALVES					
Calyptogena magnifica					
mantle	RG	-32.0 (2?)	+2.4	(2?)	Rau 1981a,b; 1985
mantle	CA	-32.6 (2?)	+4.0	(2?)	Rau 1981a,b; 1985
"rest"	**RG**	**-32.6±0.5 (27)**	**+4.5±1.3**	**(10)**	**Fisher et al. 1988c**
gill	RG	-33.2±0.7 (27)	+1.8±1.3	(10)	Fisher et al. 1988c
Calyptogena phaseoliformis					
mantle	**GR**	**-35.9 (1)**	**-10.1**	**(1)**	**this report**
Vesicomya gigas					
1/2 of specimen	**GB**	**-35.8 (2)**	**-1.0**	**(2)**	**this report**
Bathymodiolus thermophilus					
"tissue"	MB	-33.5 (3)			Williams et al. 1981
mantle and foot	CB	-33.2 (1)			Rau & Hedges 1981
foot	RG	-32.9 (1)	+1.7	(1)	Van Dover & Fry Submitted
"rest"	**RG**				
central population		**-34.4±0.8 (28)**			**Fisher et al. 1988b**
peripheral population		-33.5±0.8 (11)			Fisher et al. 1988b
"gill"	RG				
central population		-35.7±0.7 (28)			Fisher et al. 1988b
peripheral population		-34.7±0.9 (11)			Fisher et al. 1988b
"tissue"	**RG**				
central population			**-3.9±0.6**	**(6)**	**Fisher et al. 1988b**
peripheral population			+3.5±0.7	(8)	Fisher et al. 1988b

Table 2. Continued.

Bathymodiolus sp.1						
foot and adductor	11N	-34.2	(1)	-12.0	(1)	this report
Bathymodiolus sp. 2						
mantle	MA	-32.8	(1)	-0.5	(1)	Van Dover & Fry Submitted
gill	MA	-34.8	(1)	-3.0	(1)	Van Dover & Fry Submitted
SEEP BIVALVES						
Vesicomya gigas						
"body"	NC	-36.3	(2)			Kennicutt et al. In Press
gill	NC	-36.2	(2)			Kennicutt et al. In Press
Calyptogena ponderosa						
"soft tissue"	LA	-35.4	(2)			Kennicutt et al. 1985
"tissue"	LA	-36.9 to -39.1	(3)			Brooks et al. 1987
"soft tissue"	LA	-34.9	(2)	+1.1 to +7.1	(3)	Brooks et al. 1987
average		-36.6		+4.1		
Calyptogena phaseoliformis						
mantle	JT	-38.1	(1)	+3.3	(1)	Boulegue et al. 1987
mantle	JT	-36.7	(1)	-0.8	(1)	Saino & Ohta In Press
average		-37.4		+1.2		
gill	JT	-40.1	(1)	-2.8	(1)	Boulegue et al. 1987
gill	JT	-38.7	(1)	-3.3	(1)	Saino & Ohta In Press
Calyptogena soyae						
mantle	SB	-35.0	(1)	-6.1	(1)	Saino & Ohta In Press
gill	SB	-35.3	(1)	-9.6	(1)	Saino & Ohta In Press
Calyptogena sp. 1						
foot, adductor	FL	-37.1	(1)	-5.9	(1)	this report
tissue ???	FL	-36.7	(3)			Cary et al. Submitted
average		-36.9				
Calyptogena sp. 2						
foot, mantle	LF	-34.7	(1)	-1.8	(1)	this report
Calyptogena sp. 3						
gill	OR	-51.6	(1)			Kulm et al. 1986
Vesicomya cordata						
"soft tissue"	OR	-36.3	(1)	-0.9	(1)	Kulm et al. 1987
Bathymodiolus sp. 3						
tissue	FL	-74.3±2.0	(10?)	-8.9	(2?)	Paull et al. 1985
mantle	FL	-73.0±2.2	(5)			Cary et al. Submitted
gill	FL	-72.9±1.1	(5)			Cary et al. Submitted

Table 2. Continued.

Bathymodiolus sp. 4				
??	LA	-40.1 to -57.6 (38)		Brooks et al. 1987
soft tissue	LA	-45.5 to -50.1 (9)	-12.9 to +3.0 (10)	Brooks et al. 1987
mid-point			-5.0	
mantle	LA	-53.9±2.9 (3)		Childress et al. 1986
gill	LA	-51.8±0.2 (3)		Childress et al. 1986

Table 3. Non-isotopic evidence for energy sources in vent and seep symbionts. EPR = East Pacific Rise hydrothermal vents; GSC = Galapagos Spreading Center hydrothermal vents; FL = Florida Escarpment seeps; LA = Louisiana Seeps; JT = Japan Trench seeps; NC = Northern California seeps.

Species	Locale	Energy Source	Evidence	References
R. pachyptila	EPR GSC	H_2S	RuBPCase, ATP sulfurylase, APS reductase, rhodanase activity in trophosome	Felbeck 1981 Felbeck et al. 1981 Fisher et al. 1988a
			High S^o concentrations in trophosome	Cavanaugh et al. 1981 Fisher et al. 1988a
			Lack of uptake of CH_4	Childress et al. 1984
			$^{14}CO_2$ uptake and incorporation into malate and succinate	Felbeck 1985
			Sulfide-binding proteins present	Arp & Childress 1983
		[amino acids]	Uptake of amino acids	Childress et al. 1984
E. laminata	FL	H_2S	High S^o concentrations in trophosome; sulfide oxidase activity; circulating sulfide binding proteins	Cary et al. Submitted
Escarpia-like	LA	H_2S	$^{14}CO_2$ fixation by trophosome preps	Fisher et al. In Press
C. magnifica	EPR	H_2S	RuBPCase, ATP sulfurylase, APS reductase, rhodanase activity in gills	Felbeck et al. 1981 Fisher et al. 1988c
			Vestigial ciliary groove, reduced labial palps, reduced digestive tube, empty guts;	Boss & Turner 1980 Fiala-Medioni & Metivier 1986
			High S^o concentrations	Childress & Mickel 1982 Fisher et al. 1988c
			No significant uptake of CH_4; sulfide-binding protein present	Arp et al. 1984
			Sulfide oxidase activity	Fisher et al. 1988c

Table 3. Continued.

C. ponderosa	LA	H_2S	Absence of "Type 1" methane-oxidizing bacteria in gills; high S^o in gills	Brooks et al. 1987
C. phaseoliformis	JT	CH_4 or H_2S	CH_4 and H_2S in pore waters	Boulegue et al. 1987 Saino & Ohta In Press
		H_2S	S^o crystals in gills; Absence of "Type 1" methane-oxidizing bacteria in gills	Chassard-Bouchaud et al. 1988 Fiala-Medioni & Le Pennec 1986 Fiala-Medioni & Le Pennec 1988
V. gigas	NC	H_2S	High S^o concentration in gills	Kennicutt et al. In Press
B. thermophilus	EPR GSC	H_2S	RuBPCase, ATP sulfurylase, APS reductase, rhodanase activity in gill tissues	Felbeck et al. 1981 Fisher et al. 1988b
		[Thiosulfate	CO_2 fixation in presence of thiosulfate; Appreciable levels of thiosulphate in tissues]	Belkin et al. 1986 Fisher et al. 1988 b
		H_2S and particulates	Bacteria and diatoms in gut; simplified but functional mouth and gut	Le Pennec & Prieur 1984 Le Pennec et al. Hily et al. In Press
			Lack of S^o in gills; Low level of RuBPCase and sulfide oxidase activity in gills	Fisher et al. 1987 Fisher et al. 1988b
		Dissolved amino acids	Uptake and incorporation of 3H amino acids through gills	Fiala-Medioni et al. 1986

Table 3. Continued.

Bathymodiolus sp. 3	FL	CH_4	"Type 1" methane-oxidizing bacteria in gills; methanol dehydrogenase activity; hexulose phosphate synthase activity; absence of RuBPCase activity	Cavanaugh et al. 1987 Cary et al. Submitted
Bathymodiolus sp. 4	LA	CH_4	Methane consumption in gill tissues.	Childress et al. 1986
			Mussel growth on methane.	Cary et al. 1988
			"Type 1" methane-oxidizing bacteria in gills; ^{14}C-methane uptake and appearance of label in CO_2 and organic compounds; methanol dehydrogenase activity in gills; absence of ATP sulfurylase and APS reductase activity.	Fisher et al. 1987

FIGURE LEGENDS

Figure 1. Map showing the locations of vents (•) and seeps (o) discussed in this paper.

VENTS
1: Galapagos Spreading Center
2: 11°N, East Pacific Rise
3: 21°N, East Pacific Rise
4: Guaymas Basin
5: Gorda Ridge
6: Juan de Fuca Ridge
7: Marianas Back Arc Spreading Center

SEEPS
8: Laurentian Fan
9: Florida Escarpment
10: Louisiana Seeps
11: Oregon Seeps
12: N. California Seeps
13: Japanese Seeps

Figure 2. $\delta^{13}C_{PDB}$ vs. $\delta^{15}N_{AIR}$ for vent and seep symbionts.

Figure 3. Summary of expected ranges of values of $\delta^{13}C$ under various nutritional strategies and of observed ranges of values of $\delta^{13}C$ at vents and seeps. See text for discussion.

Figure 4. Summary of expected ranges of values of $\delta^{15}N$ under various nutritional strategies and of observed ranges of values of $\delta^{15}N$ at vents and seeps. See text for discussion.

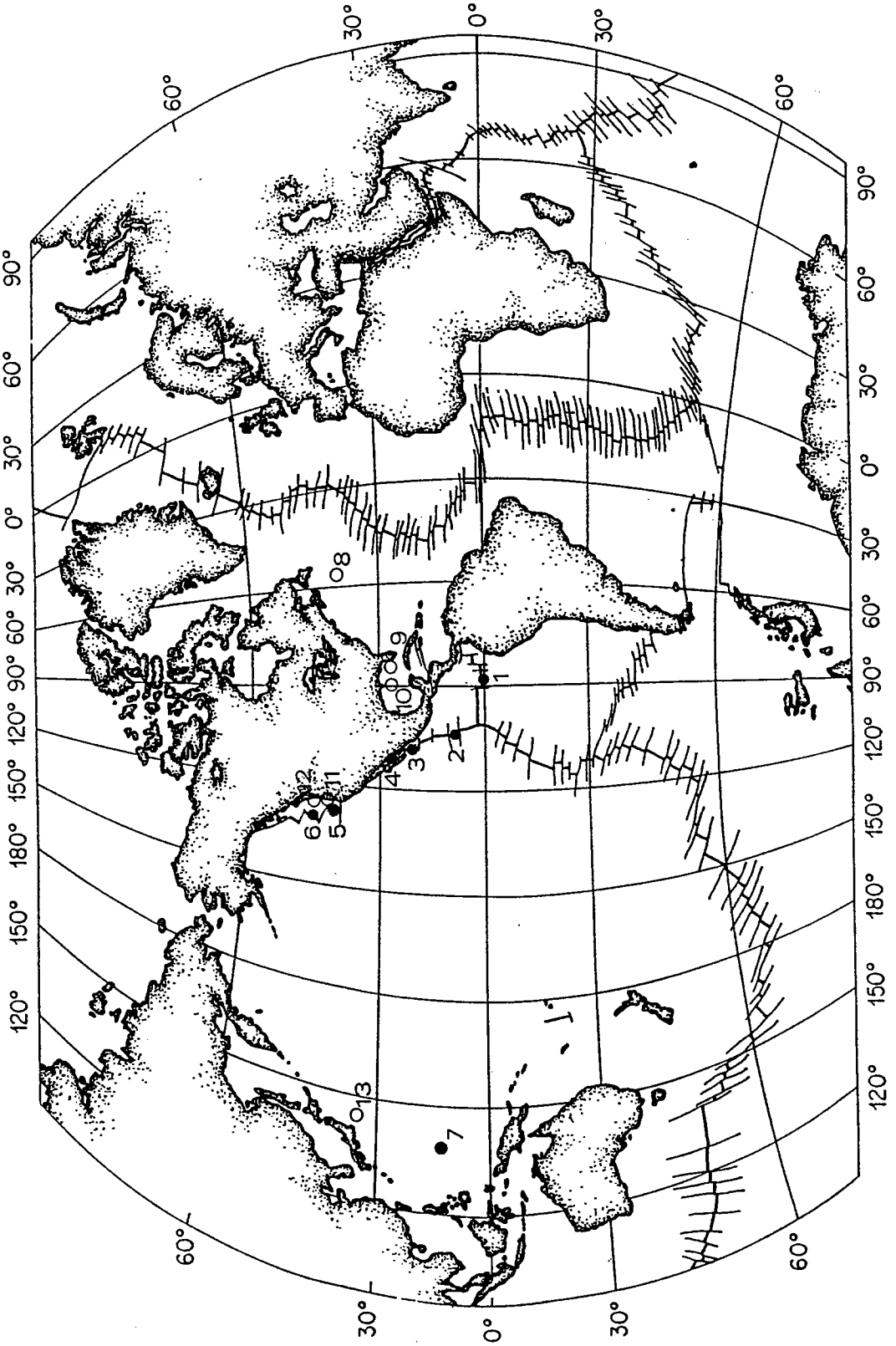

Figure 1

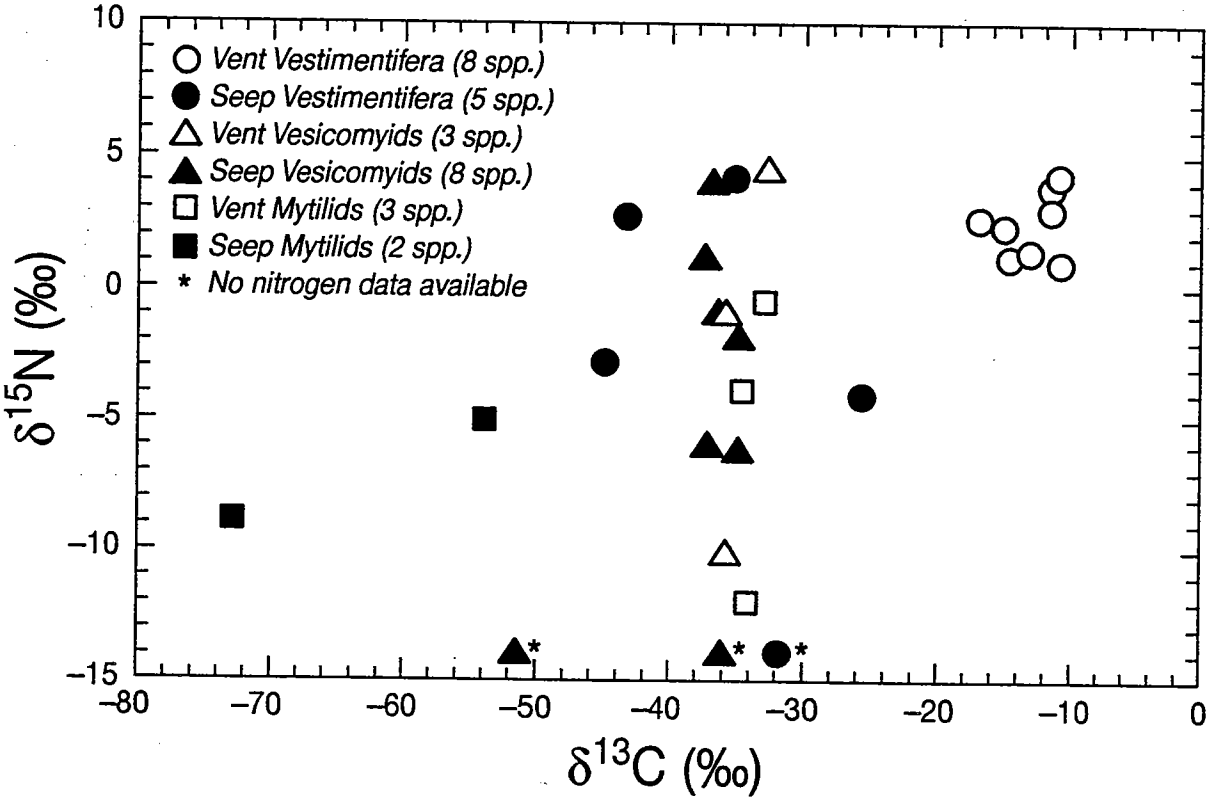
Vent Vestimentifera (8 spp.)
Seep Vestimentifera (5 spp.)
Vent Vesicomyids (3 spp.)
Seep Vesicomyids (8 spp.)
Vent Mytilids (3 spp.)
Seep Mytilids (2 spp.)
* No nitrogen data available
δ15N (‰)
δ13C (‰)
10
5
0
−5
−10
−15
−80
−70
−60
−50
−40
−30
−20
−10
0

Figure 2

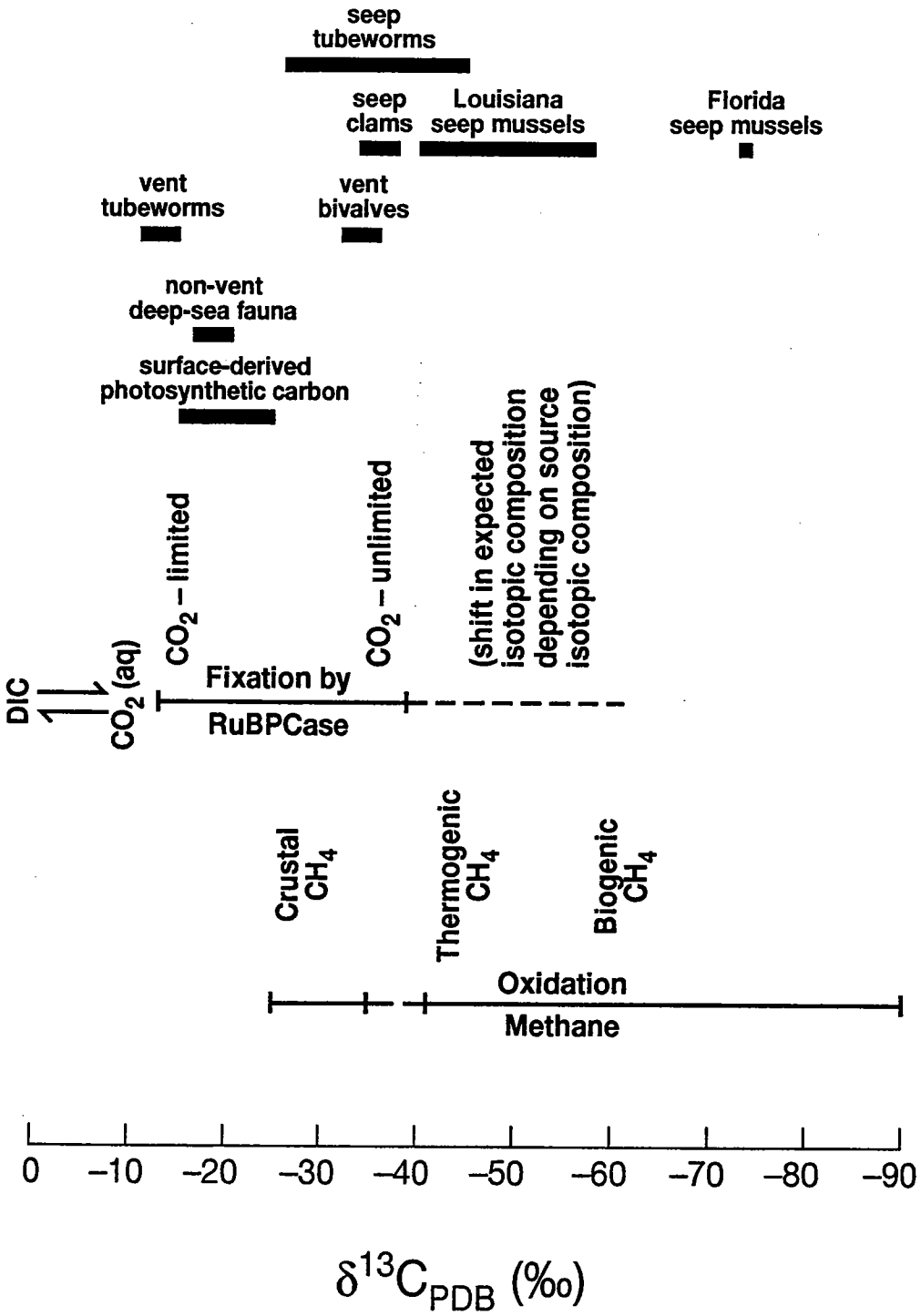
seep tubeworms
seep clams
Louisiana seep mussels
Florida seep mussels
vent tubeworms
vent bivalves
non-vent deep-sea fauna
surface-derived photosynthetic carbon
CO2 – limited
CO2 – unlimited
(shift in expected isotopic composition depending on source isotopic composition)
DIC
CO2 (aq)
Fixation by RuBPCase
Crustal CH4
Thermogenic CH4
Biogenic CH4
Oxidation Methane
0 –10 –20 –30 –40 –50 –60 –70 –80 –90
δ13CPDB (‰)

Figure 3

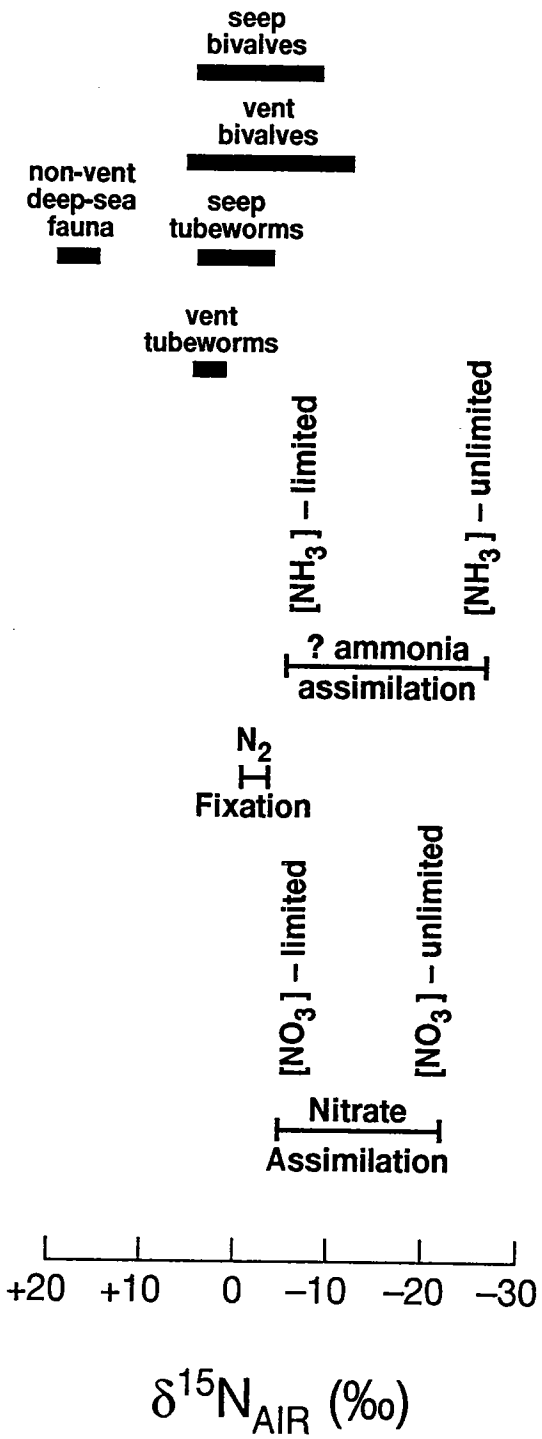
seep bivalves
vent bivalves
non-vent deep-sea fauna
seep tubeworms
vent tubeworms
[NH3] – limited
[NH3] – unlimited
? ammonia assimilation
N2 Fixation
[NO3] – limited
[NO3] – unlimited
Nitrate Assimilation
+20
+10
0
–10
–20
–30
δ15NAIR (‰)

Figure 4

Chapter 7

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Marine Biology

Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge

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Abstract

A newly described species of shrimp, *Rimicaris exoculata* Williams and Rona, 1986, dominates the megafaunal community at two hydrothermal vent sites on the Mid-Atlantic Ridge. Behavioral observations and gut-content analyses indicate that these shrimp ingest large amounts of sulfide particles from black smoker chimneys. We found no evidence for chemoautotrophic endosymbionts in *R. exoculata*, based on analyses of morphology, stable isotopes, lipopolysaccharides, and ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity. Instead, we suggest that the shrimp are normal heterotrophs, grazing on free-living microorganisms associated with black smoker chimneys. We infer that high bacterial productivity is required to sustain populations of *R. exoculata* at these vent sites.

Introduction

Hydrothermal vent and cold-water seep communities in the deep sea have been characterized by an abundance of large, relatively sessile megafauna. Tubeworms, clams, and mussels that harbor symbiotic, chemoautolithotrophic bacteria typically dominate these communities (reviewed by Grassle 1986). In contrast, tubeworms were not observed at newly discovered hydrothermal vent communities on the Mid-Atlantic Ridge (MAR) and bivalve molluscs are rare (Rona 1985, Detrick et al. 1986a, b, Grassle et al. 1986, Rona et al. 1986). These sites, however, do possess a distinctive fauna dominated by active, motile swarms of caridean shrimp. The shrimp belong to a new genus, *Rimicaris*, and two new species, *R. exoculata* and *R. chacei*, in the family Bresiliidae (Williams and Rona 1986).

Both *Rimicaris exoculata* and *R. chacei* appear to be widely distributed along the Mid-Atlantic Ridge (MAR). They have been found at the TAG and Snake-Pit hydrothermal sites (Williams 1987), areas separated by 307 km and the Kane Fracture Zone. At these sites, *R. exoculata* was by far the more abundant species in the material collected. This shrimp, 4 to 5 cm in total length, has a distinctive morphology, with enlarged antennal and antennular peduncles, no rostrum, no lenses or eyestalks, and a carapace that wraps almost entirely around the animal, creating a nearly tubular branchial chamber on either side (Williams and Rona 1986). The gills of *R. exoculata* are of typical external morphology, but the exopods of the first maxillipeds and the second maxillae are atypical, being enormously expanded (Williams and Rona 1986).

Chemical analyses of black smoker waters indicate a qualitative similarity between MAR and East Pacific Rise hydrothermal effluents (Edmond et al. 1986), including elevated hydrogen sulfide concentrations. Previous studies of deep-sea vent communities suggest that their food webs are based on primary production by chemoautotrophic prokaryotes that use hydrogen sulfide as an energy source (Karl et al. 1980, Cavanaugh et al. 1981, Felbeck 1981). Chemoautotrophic production is likely to be important at MAR vents as well. We studied the trophic biology of *Rimicaris exoculata* to determine how chemoautotrophic production may be linked to high shrimp-population densities.

Materials and methods

Rimicaris exoculata used in this study were collected in 1985 by dredging at the TAG hydrothermal field (26°08'N, 44°49'W; 3 630 m) in the rift valley of the Mid-Atlantic Ridge. A portion of the dredged material was preserved in formalin and stored in 70% EtOH; the remainder was frozen. Video records of shrimp behaviour in situ were made at the TAG and Snake-Pit (23°22'N, 44°57'W; 3 500 m) sites in 1986 during "Alvin" Dives 1675–1677 and 1683, respectively. Observations of shrimp at TAG and

Snake-Pit sites in 1985 and 1986 indicate that shrimp populations are persistent at a given site for at least one year.

A fragment of black smoker chimney near where shrimp were swarming was collected from the TAG area on "Alvin" Dive 1675 and stored dry. Subsamples from inner, middle, and outer regions of a 2 cm cross-section of this chimney wall were acidified under vapors of concentrated HCl to remove carbonates, and were analyzed for organic carbon and nitrogen content using a CHN analyzer.

A sample of sulfide deposits from the vicinity of shrimp aggregations at the Snake-Pit site was collected using a scoop sampler on "Alvin" Dive 1683 and preserved in formalin. Subsamples were removed for scanning electron microscopy (SEM) and epifluorescence microscopy to document, qualitatively, the presence or absence of bacterial populations adhering to sulfide particles.

Material prepared for SEM was dehydrated, critical-point-dried and gold-coated. For qualitative analyses, shrimp gut-content material was carbon-coated and probed using an ETEC AutoScan SEM with an energy-dispersive KEVEX spectrometer (EDX). In addition, smear slides of shrimp gut-contents were prepared and analysed by x-ray diffraction using a Phillips 3500 spectrometer.

Direct observations of bacterial populations were made using epifluorescence microscopy of nuclear material stained with 4',6'-diamidino-2-phenylindole (DAPI), following the modified technique of Huber et al. (1985), or with acridine orange (Hobbie et al. 1977).

Assays for the presence of lipopolysaccharides (LPS), a component of the outer cell wall of gram-negative bacteria, were conducted using the *Limulus* amoebocyte lysate test (Watson et al. 1977) on dried chimney sulfides, shrimp gut contents, shrimp hepatopancreas, and shrimp gonad.

Activity of ribulose-1,5-bisphosphate carboxylase (RuBPCase), an enzyme diagnostic of CO_2 fixation, was assayed in frozen shrimp hepatopancreas and abdominal muscle collected in 1985, using published techniques (Cavanaugh 1983). Tissues from three individuals were analyzed separately. Enzyme activities in the tissues were compared to spinach and substrate-free controls.

Frozen shrimp abdominal muscle was acidified with 1 *N* HCl to remove contaminating carbonates, dried, and analyzed for carbon- and nitrogen-stable isotopic compositions following the methods of Minagawa et al. (1984). Additional shrimp abdominal muscle, pooled from five individuals, was repeatedly leached with 0.5 *M* LiCl followed by distilled water to remove inorganic sulfate. Inorganic sulfide samples from chimney material and deposits at the base of a chimney were similarly treated to remove sulfate. All samples for sulfur isotope analyses were oxidized to sulfate by Parr bomb combustion; the resulting sulfate was precipitated with barium. Barium sulfate was converted to sulfur dioxide according to the methods of Yanagisawa and Sakai (1983). CO_2, N_2 and SO_2 gases were analyzed separately with a Finnegan MAT 251 isotope-ratio mass spectrometer for isotopic determinations, expressed as ‰ differences from a standard, where:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \text{ (‰)},$$

and

$$X = {}^{13}C, {}^{15}N, \text{ or } {}^{34}S$$
$$R = {}^{13}C/{}^{12}C, {}^{15}N/{}^{14}N, \text{ or } {}^{34}S/{}^{32}S.$$

The standards used were PeeDee belemnite (PDB), air, and Canyon Diablo troilite, respectively.

Results

Feeding behavior and functional morphology of *Rimicaris exoculata*

Video observations

Shrimp behavior was recorded in situ on videotape during "Alvin" Dives at TAG and Snake-Pit hydrothermal sites. The shrimp are highly mobile and occur in swarms reaching densities of approximately 1 500 per m^2 on surfaces of black smoker chimneys. They were not observed in the peripheral regions of the vent areas where large anemones were abundant, nor beyond the periphery, on non-vent terrain. Occasional shrimp were observed in the water column as much as 100 m above the hydrothermal field. Highest densities of shrimp, where the shrimp are packed side by side and may be piled two or more deep, are always near venting hot water (~ 350 °C). Water temperatures of 20° to 30 °C have been reported within the shrimp swarms (G. Thompson personal communication), but details of the temperature gradients around the shrimp are unknown. The boundary of the main body of a swarm on a given surface is distinct (Fig. 1). When disturbed by "Alvin" maneuvers, the shrimp immediately moved back onto the area from which they had been displaced. Their persistence in occupying particular areas is so great as to interfere with "Alvin" sampling efforts (J. Edmond personal communication). Any given shrimp spends only a brief period of time – seconds to minutes – in a particular location before being displaced by another individual. Shrimp on the substrate typically do not rest quietly; instead, they are continuously active, moving over a confined area.

Functional morphology

We examined the external morphology of shrimp appendages to determine a mechanism for feeding. The dactyls (tips) of the last 3 pairs of walking legs (Pereiopods III–V) of *Rimicaris exoculata* are armed with a corneous nail and strong spines (Fig. 2A). The joints of the legs permit movement in an anterior to posterior direction. Spines on the penultimate segment (propodus) of these legs are directed down-ward and are located in two

Fig. 1. *Rimicaris exoculata.* Swarms of shrimp on a black smoker chimney, TAG hydrothermal field, Mid-Atlantic Ridge. Object on left is vertical measuring rod with 5 cm divisions. (Photograph courtesy of NOAA-WHOI-MIT Mid-Atlantic Ridge Vents Research Team)

fields of rows along the same surface as the spines of the dactyl. The first two pairs of legs (Pereiopods I and II) are short and less calcified than the walking legs. Each leg of the first pair is chelate; the chelae are scoop-shaped, with no gape between the fingers and with setae fringing the hollowed-out region (Fig. 2B). The joints of the first pair of legs allow the scoop to draw material down toward the mouth. The last segment of the endopodite of the seeond maxilliped is armed with a setal brush that fits perfectly into the scoop of the cheliped (Figs. 2C, 3). Mouthparts lying against the mouth bear dense fields of spines and setae.

There do not appear to be any extraordinary modifications in the structure of the digestive system. The foregut, consisting of esophagus, cardiac and pyloric chambers, and gland filter, resembles that of other caridean shrimp (Felgenhauer and Abele in press). The hepatopancreas, midgut and hindgut are also, superficially at least, of typical morphology. Details of the internal anatomy of the digestive system are under study by Dr. B. Felgenhauer, Florida State University.

Stomach-content analyses

All the stomachs examined ($n=35$) were filled with black metallic sulfide crystals (Fig. 4A). The sulfide material passes through the foregut and was found in midgut and hindgut regions of the digestive system.

Mineralogy

Results from analyses of gut contents of shrimp by x-ray diffraction clearly showed the presence of four minerals. Chalcopyrite ($CuFeS_2$) and pyrite (FeS_2) were the two most abundant sulfides present, with lesser amounts of sphalerite (ZnS). Traces of anhydrite ($CaSO_4$) were also identified. These results were confirmed by SEM observations and EDX analyses of individual particles. Typical grain sizes were approximately 2 μm, although fibers of anhydrite were elongate.

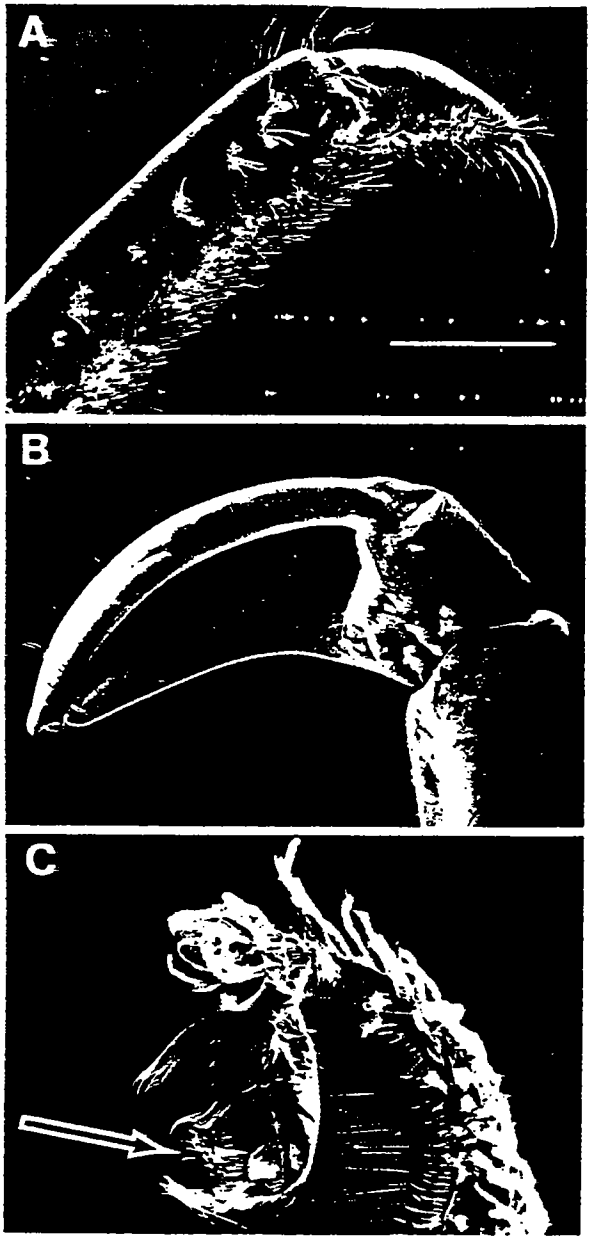

Fig. 2. *Rimicaris exoculata.* (A) Dactyl and propodus of Pereiopod III: (B) chela of Pereiopod I: (C) endopodite of Maxilliped II with arrow pointing to setal brush. Scale bar = 1 mm: (A) (B) (C) are to same scale

Organics

Direct observations (light microscopy, SEM) of stomach contents provide little indication of the organic source of nutrition. Metazoan remains (exoskeletons, setae, spicules, partially digested flesh) were not observed. DAPI staining for prokaryotes was inconclusive; under the SEM, the presence of prokaryotic cells could not be confirmed.

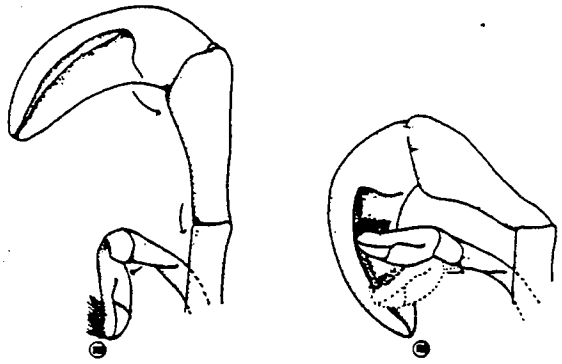

Fig. 3. *Rimicaris exoculata.* Schematic illustration of setal brush of Maxilliped II endopodite and scoop-shaped chela of Pereiopod I. Arrows indicate range of flexure; all movements at joints lie approximately in plane of the page. m: mouth

Lipopolysaccharide assays on stomach contents from two individuals indicated the presence of a large amount of bacterial cell-wall material. Based on an average heterotrophic marine bacterium LPS content of 3 fg LPS/cell (1 fg = 10^{-15} g) and an average bacterial carbon content of 19 fg C/cell (Watson et al. 1977), estimated bacterial densities associated with the cardiac stomach sulfides are on the order of 10^9 cells per ml or approximately 1 μg bacterial carbon per mg (wet weight) stomach content. LPS concentrations in control tissues (hepatopancreas and gonad) of the shrimp were below the sensitivity of the assay. Since sensitivity of the assay is on the order of 600 fg LPS/ml, control tissues had less than ~ 200 cells per ml of tissue.

Associated microflora

Filamentous, DAPI-staining microorganisms, presumably prokaryotic, densely coat the setae of the exopodites of the mouthparts of *Rimicaris exoculata*, especially of Maxilliped I and Maxilla II (Fig. 4B); similar epibionts occur on the propodal spines of Pereiopods III through V.

Results of RuBPCase enzyme assays of shrimp hepatopancreas and abdominal muscle were indistinguishable from substrate-free control values.

Substrate analyses

Organic carbon and nitrogen contents of the fragment of black smoker chimney were below detection limits for subsamples from inner and middle regions. Organic carbon content of the outer region was 0.06%; no organic nitrogen was detected in this region. Lipopolysaccharide was not detectable in 3 LPS assays of chimney material from inner, middle or outer regions. Sulfide deposits were not analyzed for organic carbon and nitrogen because they had been preserved in formalin.

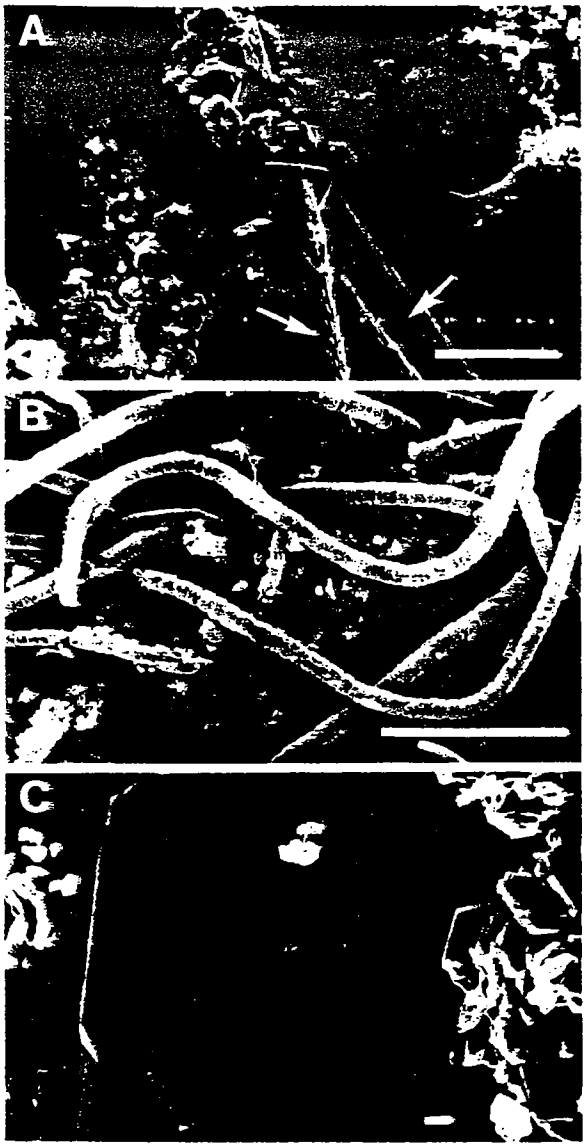

Fig. 4. *Rimicaris exoculata.* (A) Sulfide crystals in stomach (arrows point to stomach setae); (B) filamentous microorganisms on setae of Maxilla I expodite; (C) microorganisms from within matrix of sulfide crystals of hydrothermal deposits. Scale bars = 10 μm (A, B) and 1 μm (C)

Sulfide deposits collected beneath shrimp aggregations were populated by DAPI- and acridine orange-staining prokaryotes. The presence of bacteria on crystals within the matrix of the porous sulfides is documented in SEM micrographs (e.g. Fig. 4C). A transparent, amorphous, organic-looking material that stained with Rose Bengal appears to bind the sulfide crystals.

No large metazoans were observed attached to either the chimney sulfides or the sulfide deposits.

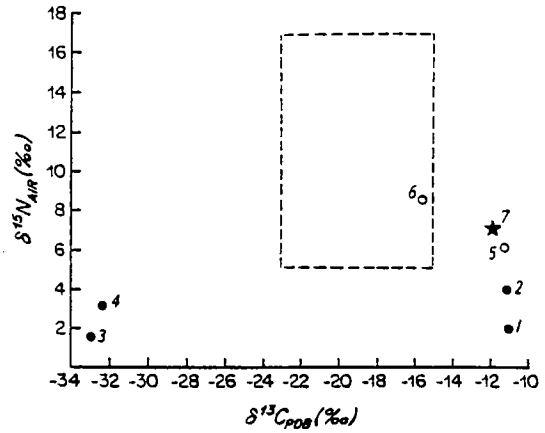

Fig. 5. $\delta^{13}C_{PDB}$ vs $\delta^{15}N_{AIR}$ (‰). Box outlines range of values reported for shallow-water marine fauna (Rau 1982, Fry and Sherr 1984). Star indicates *Rimicaris exoculata* values, filled circles species with endosymbionts. Published values for vent fauna are summarized in Rau (1985). Vestimentifera: *(1) Riftia pachyptila* trophosome, *(2) Riftia pachyptila* vestimentum; Bivalvia: *(3) Bathymodiolus thermophilus, (4) Calyptogena pacifica*; Polychaeta: *(5) Alvinella pompejana*; Crustacea: *(6) Bythograea thermydron, (7) Rimicaris exoculata*

Stable isotope analyses

For abdominal muscle of *Rimicaris exoculata*, $\delta^{13}C = -11.6$ and -12.1‰ (2 individuals), $\delta^{15}N = +7.5$ and $+7.7$‰ (2 individuals), and $\delta^{34}S = +9.7$‰ (5 pooled individuals). *R. exoculata* has $\delta^{13}C$ and $\delta^{15}N$ values similar to some Pacific vent animals (Fig. 5). The shrimp $\delta^{34}S$ values were higher than the 2.6 and 7.5‰ values measured for samples of Snake-Pit sulfide deposits and TAG chimney sulfides, respectively.

Discussion

Rimicaris exoculata occurs in dense swarms that feed on the sulfide matrix of black smoker chimneys, jockeying for position on sharply defined areas. Three lines of evidence suggest that these shrimp are normal heterotrophs that ingest food from their environment rather than relying on endosymbiotic bacteria for their nutrition:

(1) Gut contents. Analyses of material contained within the cardiac stomachs of *Rimicaris exoculata* indicate that the shrimp ingest large quantities of iron, copper, and zinc sulfides and pass them through their digestive systems. The sulfide minerals in the shrimp stomachs are typical major constituents of mineral assemblages that comprise active chimneys in the TAG and Snake-Pit areas (Thompson et al. 1986, Schroeder et al. 1986, Sulanowska et al. 1986).

While scanning electron microscopy of formalin- and glutaraldehyde-preserved stomach contents showed no

identifiable organic material. lipopolysaccharide assays reflected bacterial densities on the order of 10^9 cells per ml stomach contents. Inability to observe these bacteria directly with SEM may be a consequence of the delay in preservation of material until several hours after collection. Digestive enzymes are secreted into the cardiac stomachs of caridean shrimp (Dall and Moriarty 1983); if this is the case in *Rimicaris exoculata*, digestion of bacterial cells may be rapid.

(2) Functional anatomy. Spinous dactyls on the walking legs for scraping, scoop-shaped chelipeds for shoveling, and setose maxillipedal endopods that brush sulfides from the chelae into the basket formed by the rest of the mouthparts account for the ability of the shrimp to pick up particulate sulfide material. Gross morphology of the digestive system does not suggest any unusual modifications that might be correlated with the presence of chemosynthetic endosymbiotic bacteria.

(3) Biochemical assays. RuBPCase assays performed on selected tissues within the shrimp were negative, further suggesting the absence of chemoautotrophic endosymbionts. This negative result is not entirely conclusive, since enzyme inactivity could be due, for example, to inappropriate or prolonged storage of frozen tissues. Alternatively, it is possible that we did not identify the correct host tissue, but *Rimicaris exoculata* does not appear to have any highly vascularized organ analagous of the trophosome of tubeworms or the enlarged, fleshy gills of bivalve molluscs.

In summary, the major source of nutrition for *Rimicaris exoculata* is likely to be bacteria living on surfaces of chimney sulfides. A secondary nutritional source may be epibiotic microorganisms, presumably filamentous bacteria, which densely coat the setae of exopodites of the mouthparts (Fig. 4B). These microorganisms may be harvested by the shrimp, either incidentally or intentionally, and may be a source of nutrition. But, inasmuch as the shrimp spend so much effort scrambling for position on the sulfides and ingesting large quantities of particulate material, it seems unlikely that the filaments are the primary source of nutrition.

Organic isotope analyses of shrimp tissue confirm that the primary carbon source is non-photosynthetic. The $\delta^{13}C$ values of shrimp abdominal muscle fall outside the range of values measured in shallow-water marine fauna that are dependent on photosynthetically-derived carbon (Fig. 5). Because animals closely resemble carbon isotopic compositions of their foods within ±2‰ (Fry and Sherr 1984), we infer that the microorganisms constituting the diet of *Rimicaris exoculata* at MAR vent sites have $\delta^{13}C$ values near the −11‰ shrimp values.

Nitrogen isotopic compositions have been used to estimate the trophic positions of animals (Miyake and Wada 1967). ^{15}N enrichment is correlated with increase in trophic level in shallow-water communities (Miyake and Wada 1967), and an average increase of ~ 3 to 4‰ per trophic level appears to be characteristic of many food webs (Minagawa and Wada 1984). Vent food webs are poorly understood and the only comparative isotopic data available is from a small set of animals collected at Pacific vent sites. Pacific vent animals with endosymbionts have low $\delta^{15}N$ values of 0 to +4‰ (Rau 1985). *Rimicaris exoculata* has a +7.5‰ value, intermediate between the low 0 to 4‰ $\delta^{15}N$ values of endosymbiont-containing organisms and the +8 to +17‰ values observed for carnivorous shallow-water marine animals (Minagawa and Wada 1984, Rau 1985, Fry 1986).

The +9.7‰ $\delta^{34}S$ value for *Rimicaris exoculata* is significantly higher than the −4.7 to +4.7‰ $\delta^{34}S$ range observed for Pacific vent animals (Fry et al. 1983). This higher value possibly indicates a microbial use of both sulfate ($\delta^{34}S$ = 21‰; Rees et al. 1978) and sulfide ($\delta^{34}S$ = 2.6 to 7.5‰) at the TAG site.

We conclude from stable isotope analyses that, while the primary organic carbon source for the shrimp appears to be non-photosynthetically derived, the nitrogen isotopic compositions of shrimp tissue suggest normal heterotrophy in *Rimicaris exoculata*. Both nitrogen and sulfur isotopic compositions of shrimp tissue are distinct from those of Pacific vent animals that harbor endosymbionts.

Potential carbon sources for the shrimp, other than free-living bacteria, have not been identified. Chimney sulfides and sulfide deposits collected from areas near where shrimp were active contain no evidence of small invertebrates – no protozoans, no minute crustaceans or annelids. These sulfides, however, were dried or preserved in formalin, techniques that are inappropriate for preservation of delicate microorganisms. The water column immediately above the shrimp is unsampled: it may contain food items for the shrimp, but a reliance on nutrition from production in the water column is not consistent with our observations of shrimp behavior, anatomy, or gut contents. We cannot assess the potential for uptake of dissolved organic compounds by the shrimp.

Free-living bacteria have been implicated as direct food sources for other vent invertebrates, including calanoid copepods (Smith 1985), pompeii worms (polychaetes), and the Galápagos vent fish (Jannasch 1985). Baross and Deming (1985) cite an example of an amphipod that grazes on smoker walls, ingesting "significant amounts of pyritic material and bacteria." The biomass of these presumed primary consumers, and hence the grazing pressure on bacterial populations, does not begin to approach that of the TAG and Snake-Pit shrimp populations.

A bacterial diet for the shrimp, as suggested by LPS assays of shrimp gut-contents, would seem to require some combination of high growth rates of bacterial populations and a large standing crop of bacterial cells on the surface of the sulfide deposits. Rapid growth of bacteria isolated from hydrothermal vents has been reported, with doubling times in the laboratory of 30 to 40 min at temperatures of 85° to 90°C (Jannasch 1985). We do not know if these rates are applicable to populations of bacteria on which shrimp may feed.

Relatively large standing crops of bacterial cells on sulfides have been observed at Pacific vent sites, but only in the absence of dense populations of grazers (Jannasch and Wirsen 1981, Baross and Deming 1985). A large standing crop of bacteria on chimney surfaces at the TAG and Snake-Pit sites, at least where the shrimp are active, seems untenable given the degree to which the shrimp appear to rework the surface sulfides. Recharge of surface bacterial populations from as yet unidentified reservoirs may be implicated. Quantitative sampling of bacterial populations on the surface and within the sulfide matrix of chimneys, determination of growth rates of these bacterial populations and of their response to experimentally reduced grazing pressure (e.g. by caging-out shrimp) remain to be conducted.

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Chapter 8

Stable Isotopic Compositions of Hydrothermal Vent Organisms

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Running Header: Isotopic Compositions of Vent Fauna

ABSTRACT

We used stable isotope analyses to study trophic relationships in two communities of deep-sea hydrothermal vent organisms in the Pacific Ocean. The community at Hanging Gardens on the East Pacific Rise (21°N) is dominated by two species of vestimentiferan tubeworms; communities at Alice Springs and Snail Pits on the Marianas Back Arc Spreading Center (western Pacific) are dominated by gastropod mollusks, barnacles, and anemones. In both locations, carbon and nitrogen isotopic values of vent invertebrates are significantly different from those non-vent invertebrates collected at 11°N on the East Pacific Rise and elsewhere in the deep sea. These distinct isotopic compositions reflect local sources of organic carbon and nitrogen used by vent consumers. Many vent invertebrates lacking chemoautotrophic endosymbionts have ^{13}C-enriched values of -11 to -16 °/oo compared to values of -17 to -22 °/oo normally observed in deep-sea fauna. This suggests that a ^{13}C-enriched food source is trophically important in both vent communities. Free-living bacteria colonizing surfaces and suspended in the water column may constitute this food resource. Nitrogen isotopic analyses show that the food web of the East Pacific Rise community has more trophic levels than the Marianas vent community.

INTRODUCTION

Deep-sea hydrothermal vents support localized ecosystems whose food webs are based primarily on microbial chemosynthesis rather than photosynthesis. Chemosynthetic microorganisms live symbiotically within host tissues of clams, mussels and vestimentiferan tubeworms and use hydrogen sulfide, thiosulfate or methane as sources of chemical energy for the production of organic carbon. These bacteria can provide the major portion of the nutritional requirements of their hosts (Childress et al. 1986). Chemosynthetic and heterotrophic 'free-living' bacteria colonizing surfaces or suspended in the water column may be the food of grazing and filter-feeding invertebrates; the importance of this organic carbon pool as a source of nutrition within vent communities is unknown.

Studies of trophic relationships of vent organisms have focused on animals that harbor chemolithoautotrophic endosymbionts (reviewed by Rau 1985 and Karl 1987; Fisher et al. 1988 a,b,c). However, a variety of non-symbiont-containing species are also abundant at vents, including trophically 'conventional' filter-feeders (e.g. barnacles), grazers (gastropods, polychaetes), and scavengers (e.g. decapod crustaceans). The extent to which these organisms rely on chemosynthetic production is largely undocumented. Trophic relationships among dominant faunal components of deep-sea hydrothermal communities, including both symbiotic and heterotrophic representatives, remain relatively obscure, though attempts to draw hypothetical food webs may be found (Hessler and Smithey, 1983). We chose to use isotopic techniques to explore these relationships among a wide variety of normal heterotrophic and symbiont-containing fauna at

Hanging Gardens (21°N) on the East Pacific Rise and at two closely-spaced communities, Alice Springs and Snail Pits, on the Marianas Back Arc Spreading Center.

We employed a dual tracer approach using $\delta^{13}C$ measurements to identify important sources of carbon for consumers at vent sites and $\delta^{15}N$ measurements to determine the number of trophic levels present. Our interpretations depend on the assumption that the $\delta^{13}C$ value of a consumer corresponds to the $\delta^{13}C$ composition of its diet (DeNiro and Epstein 1978) or is heavier by about 1 °/oo (Fry and Sherr 1984). This relative fidelity of the carbon isotope signal makes it a good source indicator. Nitrogen isotopic measurements are more valuable as trophic level indicators. The $\delta^{15}N$ values of consumers are systematically heavier (by 2-4 °/oo per trophic level) than the $\delta^{15}N$ values of their diet (DeNiro and Epstein 1981; Minagawa and Wada 1984). Highest $\delta^{15}N$ values are found for top carnivores in marine systems (Rau 1982); lowest values are found for herbivores and detritivores feeding on phytoplankton and bacteria (Wada 1987; Fry 1988). We combined use of $\delta^{13}C$ and $\delta^{15}N$ data with knowledge of feeding modes to estimate food web structure among consumer species in the Pacific vent communities. We also infer carbon and nitrogen isotopic compositions of chemoautotrophic producers; our data do not allow us to identify inorganic sources of carbon and nitrogen fixed by these producers. For reference, we also measured $\delta^{13}C$ and $\delta^{15}N$ values of deep-sea consumers collected away from vent sites.

MATERIALS AND METHODS

To determine the 'background' isotopic composition of deep-sea fauna dependent on organic carbon derived from surface photosynthetic production, we analyzed specimens from non-vent, hard substrate, deep-sea environments. Collections were made on *Alvin* dives 1985-2004 on the East Pacific Rise (10°55'N to 11°55'N; 2600 m), within 10 km of known vent sites but presumed to be outside the influence of chemosynthetic production.

Animals from the Hanging Gardens hydrothermal community (20°47'N; 109°09'W; 2560 m) were collected in 1985 during *Alvin* dives 1644 and 1645. The community at this site is tubeworm-dominated, although the giant white clam, *Calyptogena magnifica* Boss and Turner is also a conspicuous element of the megafauna (Berg and Van Dover 1987). Clumps of tubeworms (*Riftia pachyptila* Jones and *Oasisia alvinae* Jones) were retrieved from the sulfide chimney at this site; a variety of macrofaunal species were analyzed from these clumps.

Marianas vent fauna was collected in 1987 from two sites, Alice Springs and Snail Pits, during *Alvin* dives 1835 to 1847 (18°11'N; 144°43'W; 3650 m). These sites are separated by about 300 m and share the same basic taxa (Hessler et al. 1988). Specimens of all conspicuously dominant species were analyzed.

Lists of the faunal components of Hanging Gardens and Marianas vents are given in Table 1. Common names, species designations where available, and assignment to a feeding guild based on analogy to shallow-water relatives are provided.

All specimens were stored frozen until prepared for isotopic analysis. Frozen tissues were thawed and acidified with 0.1 N HCl to

remove contaminating carbonates, dried, and analyzed for carbon and nitrogen stable isotopic compositions following the methods of Minagawa et al. (1984). CO_2 and N_2 gases were analyzed separately with a Finnigan MAT 251 isotope-ratio mass spectrometer for isotopic determinations, and expressed as °/oo differences from a standard, where:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3 \text{ (°/oo)}, \text{ and}$$

$$X = {}^{13}C \text{ or } {}^{15}N$$

$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N.$$

The standards used were PeeDee Belemnite (PDB) and air, respectively; based on replicate analyses, precision of measurements was $\pm$ 0.2 °/oo or better.

RESULTS

Non-Vent Deep-Sea Fauna

Carbon isotopic compositions of a variety of deep-sea benthic organisms collected at depths of 2600 m from hard substrates near 11-12°N on the East Pacific Rise ranged between -17.0 and -21.3 °/oo (Table 2). This range of values corresponds to the carbon isotopic values of particulate organic carbon and of phytoplankton at similar latitudes (Rau et al. 1982). These values may be typical for deep-sea animals at both hard and soft-bottom sites since $\delta^{13}C$ values in this range have been reported for benthopelagic fish and crustaceans from the North Central and Northeast Pacific (Williams et al. 1987), for abyssal polychaetes from the Northern Bay of Biscay (Southward et al. 1981), and for shrimp and fish in the deep Gulf of Mexico (Brooks et al. 1987).

$\delta^{15}N$ values of non-vent deep-sea fauna varied from +11.6 to +15.7 (Table 2). Low $\delta^{15}N$ values (< 0 to 6 °/oo) are reported for phytoplankton and particulate organic material of oligotrophic oceanic waters (Minagawa and Wada 1984; Saino and Hattori 1987); zooplankton values varied from 2.1 to 6.0 °/oo (Mullin et al. 1984; Minagawa and Wada 1984). Relatively heavy (more positive) values of benthic deep-sea species suggest that they have ^{15}N-enriched diets, consistent with observations by Saino and Hattori (1987) that particulate organic nitrogen (PON) in the deep Pacific has a ^{15}N-enriched composition.

Vent Communities

Vent species were typically either ^{13}C-depleted or ^{13}C-enriched relative to non-vent deep-sea species. ^{13}C-depleted values (-27.3 to -34.8 °/oo) were characteristic of mollusk species with bacterial endosymbionts. ^{13}C-enriched values (-10.4 to -11.7 °/oo) were characteristic of tubeworm species with bacterial endosymbionts; ^{13}C-enriched values (-10.2 to -15 °/oo) were also associated with a variety of non-symbiont containing vent invertebrate species. The nitrogen isotopic compositions of vent consumers, ranging from -3.0 to +12.1°/oo, were significantly lower than those of non-vent species. The lowest $\delta^{15}N$ values were measured in primary consumers, including symbiont species and species observed to graze directly on bacteria. Detailed results of the isotopic compositions of faunal components at Hanging Gardens and Marianas vents are given below.

a) Hanging Gardens Community

$\delta^{13}C$ and $\delta^{15}N$ values of symbiont-containing vestimentiferan tubeworms *Riftia pachyptila* and *Oasisia alvinae* were similar to each

other and to values previously reported for *R. pachyptila* from a Galapagos site (Table 3; Rau 1981b; Williams et al. 1981). The $\delta^{13}C$ values of tubeworms, near -11 °/oo, are ^{13}C-enriched relative to the carbon isotopic composition of non-vent species; $\delta^{15}N$ values of +2 to +4.5 °/oo in tubeworms are much lighter than $\delta^{15}N$ values of non-vent species (Table 2).

In addition to two species of tubeworms, we analyzed tissues from 11 species of vent organisms that lack symbionts but are associated with chimney sulfides at Hanging Gardens. $\delta^{13}C$ values for these species ranged from -10.7 to -16.4 °/oo (Table 3, Figure 1).

Calyptogena magnifica also occurs at Hanging Gardens. Specimens from this population were not collected; nearby populations at Clam Acres have $\delta^{13}C$ = -32.6 °/oo and $\delta^{15}N$ = +3.2 to +4.9 o/oo (Rau 1981a,b).

b) Marianas Community

A new species of gastropod, *Alviniconcha hessleri* Ohta and Okutani, colonizes vent openings at Marianas sites (Hessler et al. 1988). Stein et al. (1988) present microscopic and enzymatic evidence for a sulfide-based chemoautotrophic symbiosis in this species. Carbon isotopic compositions of *A. hessleri* muscle and gill (Table 4), ranging from -27.3 to -28.2 °/oo (muscle) and from -28.0 to -29.8 °/oo (gill), are consistent with the hypothesis of a predominantly non-photosynthetic carbon source for these organisms. *A. hessleri* carbon isotopic composition is distinctly heavier than that of bivalve mollusks with symbionts by 3 to 7 °/oo. $\delta^{15}N$ values for *A. hessleri*

(Table 3) ranged from +5.2 to +7.8 °/oo (muscle) and from +3.2 to +5.2 °/oo (gill).

The large mussel found at Marianas vents is probably closely related to the vent mussel, *Bathymodiolus thermophilus* Kenk and Wilson, known from Galapagos and East Pacific Rise hydrothermal vents (Hessler et al. 1988). $\delta^{13}C$ values of muscle and gill tissue from the Marianas mussel (Table 4) were -32.8 to -34.8 °/oo, essentially identical to that of *B. thermophilus* from Mussel Bed (Rau and Hedges 1979) and of the giant white clam, *Calyptogena magnifica*, from Galapagos and East Pacific Rise vent sites (Rau 1981b, 1985). Nitrogen isotopic compositions of the Marianas mussel (Table 4) are also similar to those of *B. thermophilus* from eastern Pacific vent sites.

Non-symbiont fauna at Marianas vents is comprised of paralvinellid polychaetes, limpets, barnacles, anemones, shrimp, and crabs. These non-symbiont-bearing invertebrates have $\delta^{13}C$ values of -11 to -17 °/oo and a smaller range of $\delta^{15}N$ values compared to Hanging Gardens species that lack symbionts (Figure 2).

DISCUSSION

Carbon Isotopic Compositions.

An unexpected feature of carbon isotopic compositions at both Hanging Gardens and Marianas sites is the occurrence of consumers with ^{13}C-enriched $\delta^{13}C$ values in the -16 to -11 °/oo range (Tables 3 and 4, Figures 1 and 2). These values in consumer tissues indicate that some carbon source at the base of the food web must have a $\delta^{13}C$ value $\geq$ -11 °/oo. At Hanging Gardens, where -11 °/oo vestimentiferan tubeworms are abundant, grazing on these animals could provide one source of -11 °/oo

carbon. Our data suggest that a second source of -11 °/oo carbon is free-living chemosynthetic bacteria. Isotopic analyses of alvinellid polychaetes together with information on their mode of nutrition support this inference. *Alvinella pompejana* Desbruyeres and Laubier lives on sulfide chimneys and is a deposit-feeder or grazer, ingesting sulfide particles and associated organic material, including bacteria (Desbruyeres et al. 1983); they do not have obvious access to tubeworm tissues. The carbon isotopic compositions of alvinellid and paralvinellid polychaetes (3 species) at Hanging Gardens and at Clam Acres (near Hanging Gardens; Desbruyeres et al. 1983) are consistently ^{13}C-enriched, spanning a range of $\delta^{13}C$ values from -9.6 to -12.8 °/oo. At the Marianas site, tubeworms are absent yet paralvinellid polychaetes have $\delta^{13}C$ values near -11°/oo (Table 4). We infer that free-living bacteria with $\delta^{13}C$ values near -11 °/oo provide the source of heavy carbon measured in alvinellid polychaete and other consumer tissues.

The occurrence of ^{13}C-enriched bacteria at vents may be widespread. At the TAG hydrothermal site on the Mid-Atlantic Ridge, an abundant shrimp (*Rimicaris exoculata* Williams and Rona) has $\delta^{13}C$ values of -11.8 o/oo, again indicating an isotopically heavy carbon source (Van Dover et al. 1988). Tubeworms have not been reported from this site. Since the shrimp stomachs were filled with sulfides and bacterial cell wall material but there was no evidence of endosymbiotic associations, Van Dover et al. concluded that the shrimp feed on free-living bacteria associated with black smoker chimney sulfides.

These observations suggest the presence of significant pools of heavy, ~-11 °/oo organic carbon in free-living bacteria associated with

high temperature sulfide vents at three faunistically distinct hydrothermal vent sites in the Atlantic and Pacific Oceans. Unfortunately, these bacteria have not yet been collected for isotopic determinations.

A priori knowledge of carbon isotopic fractionation in free-living chemosynthetic bacteria (Ruby et al. 1987) led us to expect an isotopically light pool of organic carbon (-25 to -35 °/oo) for these bacteria. The nearly 25 °/oo discrepancy between expected and inferred values of $\delta^{13}C$ in free-living bacteria requires further study. We can note, however, that ^{13}C-enriched values of -5 to -15 °/oo have been observed in many other microbial mat communities where CO_2 fixation is important (Calder and Parker 1973; Schidlowski et al. 1984). It is possible that rapid CO_2 fixation in mats at vents may lead to CO_2 limitation and isotopically heavy values for chemosynthetic bacteria.

We observed a 5 °/oo range in $\delta^{13}C$ values of Hanging Gardens consumers and a 12 °/oo range in $\delta^{13}C$ values of Marianas consumers. These ranges of values indicate that at least one other source of organic carbon must be available to consumers. Potential sources include tissues of symbiont-containing mollusks (-28 to -35 °/oo) and surface-derived photosynthetic carbon (-17 to -22 °/oo).

Nitrogen Isotopic Compositions.

The distinctly lower $\delta^{15}N$ values of all vent animals compared to non-vent animals indicate that organic nitrogen in vent systems is of local origin. Autotrophic bacteria are presumably this source of nitrogen; as indicated above, these bacteria exist both as free-living populations and as endosymbionts in host tubeworm and mollusk tissues.

Isotopic analyses of bacteria-rich tissues of symbiont-containing animals provide estimates of the $\delta^{15}N$ compositions of the endosymbiont populations. Free-living bacteria were not collected in this study, but their $\delta^{15}N$ values can be inferred by subtracting 3.4 °/oo from $\delta^{15}N$ values of animals such as alvinellid polychaetes that graze these bacterial populations (Desbruyeres et al. 1983). This inference is based on the observation that animals typically have $\delta^{15}N$ values 3.4 °/oo higher than those of their diets (Minagawa and Wada 1984).

At Hanging Gardens, bacterial symbionts of tubeworms and bivalves have estimated $\delta^{15}N$ compositions between +3.2 and +4.9 °/oo. Free-living bacterial populations have inferred $\delta^{15}N$ values that are similar, +0.4 to +3.7 °/oo, based on polychaete consumer values of +3.9 to +7.2 °/oo. If we take +3 °/oo to be an average value for autotrophic bacteria within the Hanging Gardens community and given that the highest consumer $\delta^{15}N$ value is +12.1 °/oo, we find that there are approximately 3.5 trophic levels at this site: bacterial primary producers and 2.5 trophic levels of invertebrate consumers.

An estimate of 3.5 trophic levels is consistent with observations on the feeding biology of Hanging Gardens invertebrates. Two species at Hanging Gardens have patent access to tubeworm tissues and are likely to be 'secondary consumers': small coiled gastropods can be found in large numbers on the plumes of *Riftia pachyptila* and abundant limpets (*Lepetodrilus pustulosus* McLean) graze on surfaces of *R. pachyptila* tubes. The +8.5 to +9.2°/oo $\delta^{15}N$ values of the gastropods and limpets are 4-6 °/oo higher than those of tubeworms (Table 3), reflecting the higher trophic positions of the gastropods. Another candidate for 'secondary consumer' at Hanging Gardens is the polynoid

polychaete *Lepidonotopodium riftense* Pettibone, based on relatively high $\delta^{15}N$ values of +8 to +10 °/oo. Of less certain trophic status are the polychaete *Hesiolyra bergi* Blake, the nemertean, the filter-feeding barnacle *Neolepas zevinae* Newman, and the lysianassid amphipods; nitrogen isotopic compositions of these species (5.6 to 7.3 °/oo) are consistent with either primary or secondary consumer status. The top consumer within the Hanging Gardens community is the galatheid squat lobster *Munidopsis subsquamosa* Henderson. Van Dover and Lichtwardt (1986) examined stomach contents of numerous *Munidopsis subsquamosa* from vent sites at 21°N and the Galapagos Spreading Center and found evidence for a mixed diet comprised of limpets, protozoans, polychaetes and crab larvae. The high, +12.1 °/oo $\delta^{15}N$ value of *M. subsquamosa* is consistent with its designation as top consumer.

At Marianas vents, paralvinellid polychaetes have $\delta^{15}N$ values of +8 to +9 °/oo, and we infer the $\delta^{15}N$ composition of their diet, free-living bacteria, to be about +4.6 °/oo, slightly more positive than the free-living bacteria of Hanging Gardens. $\delta^{15}N$ values of endosymbiotic bacteria associated with the gastropod (*Alviniconcha hessleri*) are similar, at +3.2 to +5.2 °/oo. Adopting a value of +5 °/oo for bacterial primary producers at Marianas vents and given the highest $\delta^{15}N$ value of +10.3 °/oo, there are 2.5 trophic levels in this community. A greater number of trophic levels could occur in this system if -3 °/oo nitrogen from the mussel, *Bathymodiolus* sp., enters the system; this is unlikely since the mussel is relatively rare (Hessler, pers. comm.) and since no higher consumers have $\delta^{13}C$ values similar to those of *Bathymodiolus* (Table 4).

Observations of the behavior of the Marianas shrimp (*Rimicaris* sp.) and the limpet indicate that these species are primary grazers within the system (R.R. Hessler, personal communication). The +8.0 to +9.1 °/oo $\delta^{15}N$ values of these species are consistent with their designation as primary consumers. Anemones and barnacles have +7.0 to +8.6 °/oo $\delta^{15}N$ values nearly identical to the shrimp and limpets, although as filter-feeders, they rely on suspended particulate material rather than surface bacteria for their nutrition. Stomach contents of the bythograeid crab include shell and periostracum fragments of the snail as well as clumps of bacterial-like organic matter comprised of ~1 μm diameter spheres. A mixed feeding strategy of grazing and scavenging or carnivory by the crab is consistent with its relatively high +8.3 to +10.3 $\delta^{15}N$ values.

Community Level Approach to Isotopic Studies.

Our results show that relatively few isotopic measurements are valuable for comparing trophic relationships within deep-sea vent communities. Carbon isotopic measurements suggest that free-living bacteria are important sources of food at both Hanging Gardens and Marianas vent communities. The nitrogen isotopic analyses show that the Marianas communities may be somewhat simpler in trophic structure than the Hanging Gardens community (2.5 vs. 3.5 trophic levels). These chemical tracer studies need to be amplified by a greater attention to collecting bacterial populations in future expeditions; many interpretations made in this paper are based on inferred isotopic compositions for these bacteria. With appropriate modification of sampling schemes, it is likely that isotopic studies will be valuable

survey tools for rapid comparison of food web structure in remote environments of the deep-sea.

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Table 1. Feeding guilds at Hanging Gardens and Marianas vents based on analogy to shallow-water analogues

Feeding Guild	Common Name
HANGING GARDENS	
Symbiotic Associations:	
Riftia pachyptila Jones	Vestimentiferan tubeworm
Oasisia alvinae Jones	Vestimentiferan tubeworm
Calyptogena magnifica Boss and Turner	Vent clam
Grazers:	
Alvinella pompejana Desbruyeres and Laubier	Alvinellid polychaete
Alvinella caudata Desbruyeres and Laubier	Alvinellid polychaete
Paralvinella grasslei Desbruyeres and Laubier	Alvinellid polychaete
Coiled Gastropod	
Lepetodrilus pustulosus McLean	Limpet mollusk
Filter-feeder:	
Neolepas zevinae Newman	Barnacle
Scavengers/Carnivores:	
Nemertean	
Hesiolyra bergi Blake	Hesionid polychaete
Lepidonotopodium riftense Pettibone	Polynoid polychaete
Lysianassid amphipods	
Top Carnivore/Scavenger:	
Munidopsis subsquamosa Henderson	Squat lobster
MARIANAS	
Symbiotic Associations:	
Bathymodiolus sp.	Mussel
Alviniconcha hessleri Ohta and Okutani	Hairy gastropod
Grazers:	
Paralvinellid	Alvinellid polychaete
Limpet	
Rimicaris sp.	Shrimp
Filter-Feeders:	
Anemone	
Barnacle	
Top Carnivore:	
Bythograeid	Crab

Table 2. Non-vent deep-sea fauna $\delta^{13}C$ and $\delta^{15}N$ values. Specific tissues analyzed are indicated.

TAXON	$\delta^{13}C$ (°/oo)	$\delta^{15}N$ (°/oo)
Coelenterata		
Hydroids		
entire, pooled	-19.7	+14.1
Anemone		
body wall	-17.1	+12.9
Mollusca		
Octopus		
tentacle	-17.0	+14.1
Echinodermata		
Holothurian		
body wall	-17.1	+14.0
Brisingid		
hepatopancreas	-19.3	+11.6
gonad	-18.2	+12.3
(same individual)		
Chordata		
Stalked Tunicate		
tunic	-21.3	+15.7

Table 3. Hanging Gardens fauna $\delta^{13}C$ and $\delta^{15}N$ values. Entire animal analyzed unless otherwise specified

TAXON	$\delta^{13}C$ (°/oo)	$\delta^{15}N$ (°/oo)
Vestimentifera		
Riftia pachyptila		
vestimentum	-11.7	+4.5
trophosome	-11.3	+3.4
(same individual)		
Oasisia alvinae		
vestimentum	-11.4	+2.9
trophosome	-10.4	+3.4
(same individual)		
Gastropoda		
Lepetodrilus pustulosus	-12.0	+9.2
Globose coiled	-13.4	+8.5
Polychaeta		
Alvinella caudata		
branchiae and oral tentacles	-12.8	+3.9
body wall (same individual)	-----	+6.3
Alvinella pompejana		
branchiae and oral tentacles	-11.7	+4.7
Paralvinella grasslei	-12.8	+7.3
Hesiolyra bergi	-10.7	+7.3
Lepidonotopodium riftense	-11.6	+8.1
(2 individuals)	-12.3	+10.2
Nemertean	-11.4	+5.6
Crustacea		
Cirripedia		
Neolepas zevinae	-15.4	+5.9
(2 individuals)	-14.7	+6.5
Amphipoda		
Lysianassidae (~100 pooled)	-14.1	+7.5
Anomura (F. Galatheidae)		
Munidopsis subsquamosa abdominal tissue	-16.4	+12.1

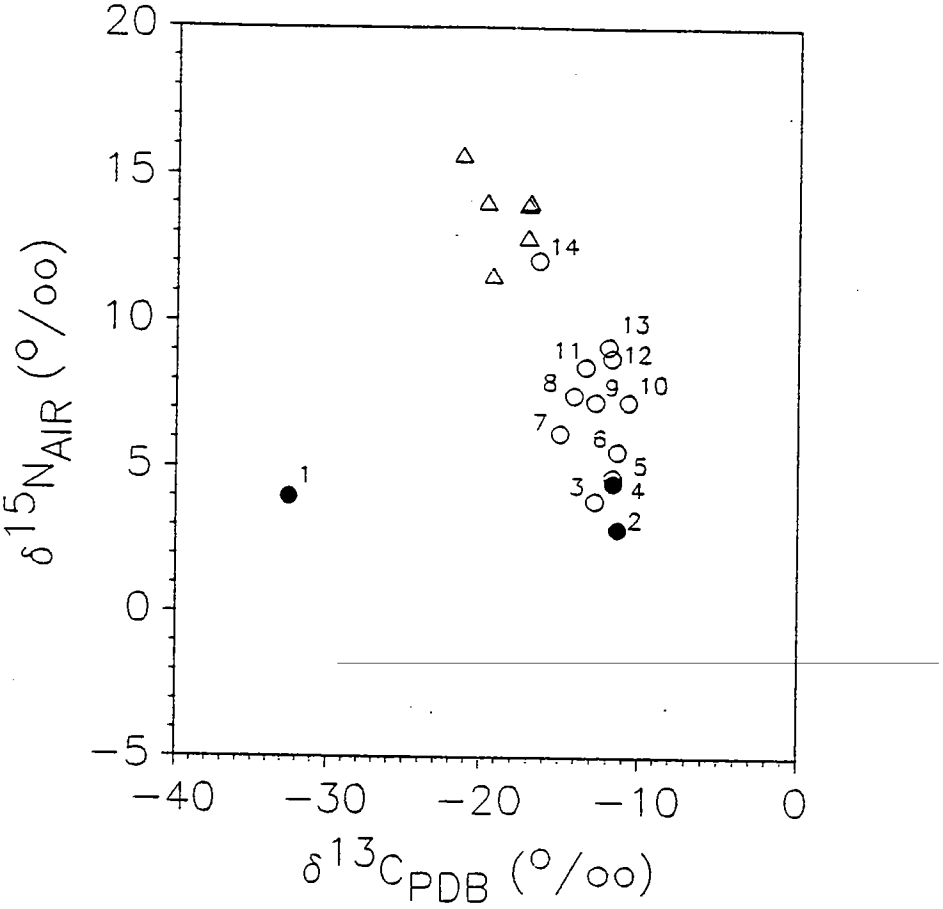
Table 4. Marianas fauna $\delta^{13}C$ and $\delta^{15}N$ values. SP = Snail Pit; AS = Alice Springs. Entire animal analyzed unless specific tissue is noted.

TAXON	LOCALE	$\delta^{13}C$ (°/oo)	$\delta^{15}N$ (°/oo)
Gastropoda			
Alviniconcha hessleri			
muscle	SP	-28.1	+7.8
gill	SP	-29.7	+3.8
muscle	SP	-27.9	+5.2
gill	SP	-29.7	+3.2
muscle	AS	-27.3	+5.7
gill	AS	-28.0	+5.2
muscle	AS	-28.2	+5.6
gill	AS	-28.3	+4.6
(two individuals from each site analyzed)			
Limpet	AS	-14.1	+8.0
	AS	-12.9	+9.1
Bivalvia			
Bathymodiolus sp.			
muscle	SP	-32.8	-0.5
gill	SP	-34.8	-3.0
(same individual)			
Coelenterata			
Anemone			
body wall	AS	-15.7	+8.6
Polychaeta			
Paralvinella sp.	AS	-10.2	+8.4
	AS	-11.8	+7.9
Crustacea			
Cirripedia			
Barnacle	SP	-23.0	+7.2
	SP	-21.8	+7.0
	AS	-15.5	+8.5
	AS	-16.0	+7.6
(four individuals analyzed)			
Macrura			
Rimicaris sp.			
abdominal muscle	SP	-16.7	+8.9
	SP	-16.4	+8.6
(two individuals analyzed)			
Brachyura			
F. Bythograeidae			
claw	SP	-14.3	+10.3
	SP	-14.7	+9.4
	AS	-14.8	+8.3
	AS	-17.2	+9.3
(four individuals analyzed)			

FIGURE LEGENDS

Fig. 1. Hanging Gardens vent community $\delta^{13}C$ vs. $\delta^{15}N$. Δ = non-vent deep-sea fauna; ● = vent species with endosymbionts; o = vent species without endosymbionts. Where more than one individual was analyzed, points represent average values. 1:*Calyptogena magnifica* (Clam Acres; Rau 1981 a,b); 2:*Riftia pachyptila*; 3:*Alvinella caudata;* 4:*Oasisia alvinae*; 5:*Alvinella pompejana*; 6:nemertean; 7:*Neolepas zevinae*; 8:Lysianassid amphipod; 9:*Paralvinella grasslei*; 10:*Hesiolyra bergi*; 11:*Lepidonotopodium riftense*; 12:*Lepetodrilus pustulosus*; 13:coiled gastropod; 14:*Munidopsis subsquamosa*

Fig. 2. Marianas vent community $\delta^{13}C$ vs. $\delta^{15}N$. Δ = non-vent deep-sea fauna; ● = vent species with endosymbionts; o = vent species without endosymbionts. Where more than one individual was analyzed, points represent average values. 1:*Bathymodiolus sp.* (gill); 2:*Bathymodiolus* sp. (muscle); 3:*Alviniconcha hessleri* (gill); 4:*Alviniconcha hessleri* (foot); 5:barnacle (Snail Pit); 6:*Rimicaris* sp.; 7:anemone; 8:barnacle (Alice Springs); 9:F. Bythograeidae; 10:limpet; 11:*Paralvinella* sp.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$\delta^{15}N_{AIR}$ (°/oo)
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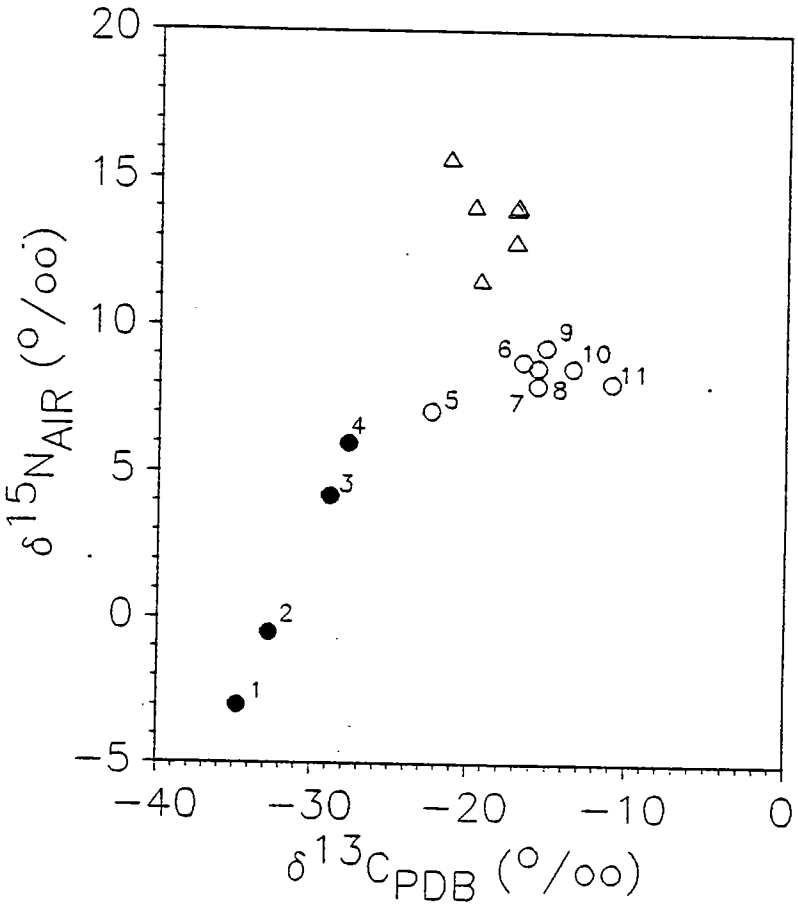
Figure 1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1
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Figure 2

PART III

VISION AND LIGHT

Chapter 9

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A novel eye in 'eyeless' shrimp from hydrothermal vents of the Mid-Atlantic Ridge

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Rimicaris exoculata[1] **is a shrimp that swarms over high-temperature (350 °C) sulphide chimneys at Mid-Atlantic Ridge hydrothermal fields (3,600 m)[1–7]. This shrimp lacks an externally differentiated eye[1], having instead a pair of large organs within the cephalothorax immediately beneath the dorsal surface of the transparent carapace, connected by large nerve tracts to the supraesophageal ganglion. These organs contain a visual pigment with an absorption spectrum characteristic of rhodopsin. Ultrastructural evidence for degraded rhabdomeral material suggests the presence of photoreceptors. No image-forming optics are associated with the organs. We interpret these organs as being eyes adapted for detection of low-level illumination and suggest that they evolved in response to a source of radiation associated with the environment of hydrothermal vents.**

Instead of tubeworms, clams and mussels that are characteristic of vent sites on the East Pacific Rise, the fauna of Atlantic vents is dominated by shrimp. Several species coexist, but *Rimicaris exoculata* is by far the most abundant. As indicated by its specific name, *R. exoculata* lacks eyestalks and corneas[1]. Instead, the shrimp possesses a pair of highly reflective patches on the dorsal surface of the cephalothorax. Dissection shows these patches correspond to an anteriorly fused, bilateral pair of specialized organs (Fig. 1), interpreted here as being novel eyes. The dorsal quarter of each eye is a compact zone of textured tissue which gives way ventrally to a fibrous web. Fibres of this web coalesce ventromedially into a nerve trunk that enters the fused supraesophageal ganglion in the same position as the optic nerve of other decapods[8]. Examination of 15-μm serial sections of paraffin-embedded tissue stained with haematoxylin and eosin reveals that each fan is composed of ommatidia-like clusters of photoreceptors (5–7 cells per cluster; average, 6). There is no evidence for a lens or dioptric apparatus associated with each 'ommatidium'. Long axes of these 'ommatidia' are oriented perpendicularly to the carapace along the dorsal surface of the organ. Each shrimp has about 9×10^3 receptor cells.

The distal portion of each photoreceptor is a cylindrical rhabdomeral segment filled with microvillar membrane; the proximal portion is a highly attenuated arhabdomeral segment that contains the nucleus (Fig. 2*a*, left arrowhead). The ultrastructure of the rhabdomeral segment (Fig. 2*b*) is almost certainly degraded from the living state[9–16] because the animals were fixed only after exposure to light at the ocean's surface. Nonetheless, the arrays of membranous vesicles and whorls are suggestive of broken-down microvillar rhabdom as found in other invertebrate photoreceptors[12–16]. The reflective property of the organ suggests a tapetal mechanism for increased light absorption by the receptors. As the reflective property is not

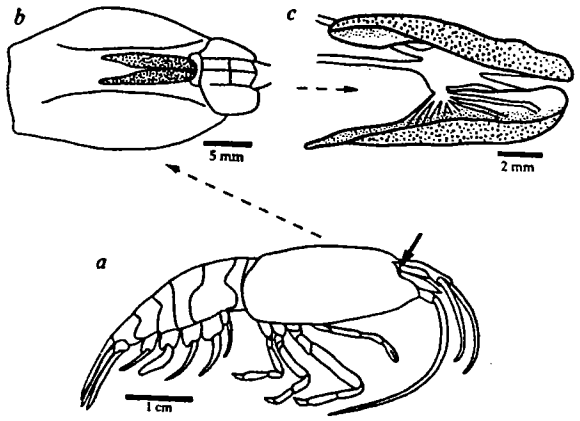

Fig. 1 *Rimicaris exoculata*. *a*, Lateral view. *R. exoculata* lacks eyestalks and conventional compound eyes. Solid arrow points to the location of eyes in the congeneric species *Rimicaris chacei*[1]. *b*, Oblique dorsal view showing the location of the novel visual organ (stippled area) underlying the thin transparent carapace. *c*, Dissection of the thoracic eye. The fused anterior tips have been separated along the midline to reveal the underlying connections to the supraesophageal ganglion.

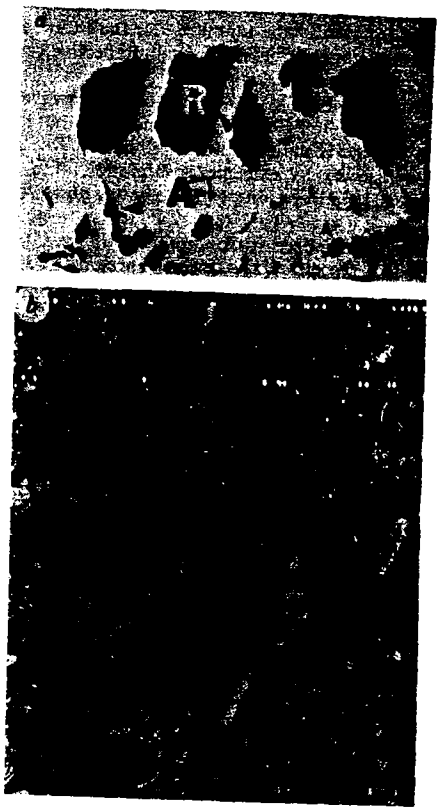

Fig. 2 Structure of photoreceptors of *Rimicaris exoculata*. *a*, Plastic 1-μm section stained with toluidine blue and cut normal to the eye surface. The rhabdomeral segment (R) is filled with a large elaboration of rhabdomere. The arhabdomeral segment (A) is severely attenuated (right arrowheads). The left arrowhead shows a photoreceptor nucleus. No other cellular elements are present. *b*, Electron micrograph of a region of rhabdomeral segment cytoplasm. The vesicles and whorls of membrane probably represent degraded microvilli from the rhabdom. The bar in *b* represents 13 μm in *a* and 0.5 μm in *b*.

preserved in frozen or fixed specimens, we cannot determine the chemical nature of the tapetum, nor the extent to which it contributes to image-formation, if at all. Reflective cells[10–12] and extremely attenuated arhabdomeral segments[12] have been demonstrated in other crustaceans adapted to low ambient light levels. Thus, morphological evidence indicates that the unusual organs in *Rimicaris* are modified compound eyes, specialized for high sensitivity by extreme proliferation of rhabdomeral membrane, deletion of a dioptric apparatus, and presence of a tapetum.

Any organ specialized for photoreception should contain a rhodopsin-like protein. We performed rhodopsin assays on the available light-adapted specimens. Rhabdomeral membranes were partially purified by either isopycnic centrifugation on sucrose gradients[17] or differential centrifugation, and pigment was extracted from the membranes with digitonin. In the presence of hydroxylamine (NH_2OH), light bleaches a shrimp pigment that maximally absorbs at 500 nm and produces a product that maximally absorbs at 366 nm (Fig. 3). The difference spectrum closely resembles that of vertebrate rhodopsins. The location, relative magnitude and shape of the absorption bands indicate that the shrimp pigment is rhodopsin and that the product of the light-induced reaction is all-*trans*-retinaloxime. Formation of all-*trans*-retinaloxime, whose absorption peak is at 365–367 nm (ref. 17), indicates that before irradiation the chromophore is retinal, and is in a *cis*-isomer configuration[17]. Formation of retinaloxime also indicates that metarhodopsin is unstable to hydroxylamine under our experimental conditions. In this respect, pigment from *R. exoculata* resembles visual pigments of pelagic euphausiids[18].

Assuming the usual extinction coefficient for rhodopsin[17], 25 pmol pigment was extracted from each organ (50 pmol per shrimp) in the experiment shown in Fig. 3. This is a lower limit, because it ignores the fraction of pigment in the metarhodopsin state and because the limited amount of shrimp tissue prevented us from optimizing experimental conditions. Compared with crayfish[19] and horseshoe crabs[20], the eyes of *R. exoculata* contain at least 2–7 times more visual pigment.

Rhodopsin in *R. exoculata* absorbs maximally at a slightly longer wavelength than has been reported for other deep-sea-dwelling crustacea. For example, visual pigments of two species of pelagic euphasiids peak at 485–488 nm in digitonin extracts[18] and in mesopelagic decapods, λ_{max} varies from 485 to 495 nm by microspectrophotometry[21]. Some of these shrimps were also studied with electrophysiological techniques and showed maximal spectral sensitivity at about 500 nm (ref. 22).

Presence of a visual pigment supports morphological evidence that the thoracic organ is an eye; the unusual nature of this eye suggests it has a novel function. In the typical deep sea at depths of 3,600 m, bioluminescence is the only known source of ambient light. Macroorganisms produce flashes of relatively high-intensity light[23] with emission maxima clustered at around 460–490 nm[22]. In general, the λ_{max} of a visual pigment closely matches the spectral distribution of the ambient light. So it is tempting to relate the visual pigment, with its λ_{max} of 500 nm, to detection of bioluminescent phenomena by the shrimp. But then why should a new sensory organ evolve in place of a normal crustacean eye which is an adequate detector of visible light for most pelagic decapods?

The dominant physical features of the shrimps' environment are plumes of water at 350 °C. Detection of plumes could attract shrimp to feeding areas[7] and deter them from plunging into water hot enough to cook them. In a medium that does not transmit infrared radiation and in an environment of very steep thermal gradients (>100 °C per cm), detection of plumes with temperature-sensitive receptors is probably inadequate. Can *Rimicaris* 'see' plumes of hot water? Visual detection might be possible if the plumes emit light. Sources of light could be chemical (as in oxidation-reduction reactions or thermoluminescence) or physical (for example, Cherenkov radiation from radioisotopes or thermal black-body radiation). Of these possibilities, only thermal radiation from 350 °C water is a certain source of visible light.

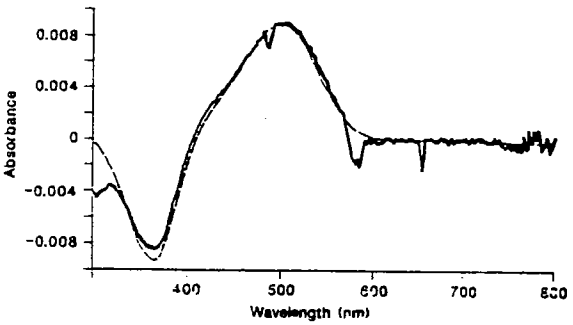

Fig. 3 Bleaching difference spectrum of visual pigment in *Rimicaris exoculata* (solid trace), obtained by subtraction of spectra measured before and after bleaching. Absorption due to reactants is positive and to products is negative. Bleaching destroys a shrimp pigment that maximally absorbs at 500 nm, typical of 'classical' rhodopsin, and creates a new pigment with a λ_{max} at 367 nm, which corresponds to retinaloxime. The same features are also seen in the difference spectrum of frog rhodopsin (dashed line) normalized to maximum absorbance at 500 nm. The slight mismatch in amplitudes of retinaloxime products may be attributed to a slightly greater (1.08 times) extinction coefficient for shrimp rhodopsin. Spikes at 660, 580 and 490 nm are instrumentation artefacts that regularly appear at these wavelengths.

Methods. All experiments were performed under dim red light with light-adapted animals. Organs of six thawed shrimps were dissected and homogenized in a solution of artificial cytoplasmic medium (80 mM NaCl, 335 mM Kisethionate, 200 mM sucrose, 4 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES titrated to *p*H 7.4 with NaOH)[26] including protease inhibitors (0.5 mM PMSF, 7 μg ml^{-1} of pepstatin, 50 μg ml^{-1} of leupeptin). The homogenized suspension was centrifuged for 30 min at 10,000g. The resulting pellet was resuspended in a hypotonic medium (10-fold dilution of the artificial cytoplasmic medium) and was centrifuged for 30 min at 51,000g. The final pellet was solubilized for 3 h at room temperature with 2% digitonin in amphibian saline solution (115 mM NaCl, 2.5 mM KCl, 1.5 mM $MgSO_4$, 10 mM HEPES titrated to *p*H 7.4 with 4 mM NaOH). Unsolubilized material was removed by centrifugation (2 min at 13,000g). The clear supernatant was used for subsequent analysis on a diode-array spectrophotometer (HP 8452, Hewlett-Packard). After the initial spectrum was recorded, 45 mM NH_2OH was added to the solution in the cuvette to destroy any retinochrome[27] present. Subsequently, the cuvette contents were exposed for 5 min to a light source that bleached ~95% of the rhodopsin. For about a 10-min interval both before and after bleaching, the time course of any dark reaction was monitored because the initial tissue homogenate contained relatively large concentrations of water-soluble pigments which could interfere with spectral analysis. In this experiment, the contribution of the dark-reaction was negligible and so no correction was made to the recorded bleaching difference spectrum. We obtained the same bleaching difference spectrum with rhabdoms that were purified by sucrose flotation[17]. We specifically searched the rhodopsin extract for the presence of membrane pigments that absorb in the red end of the visible spectrum. Our spectral analysis extended to 900 nm, but no pigment was detected that was preferentially sensitive to the longer wavelengths. The entire protocol from homogenization to extraction was also performed on control tissue derived from the abdomen of the shrimp, where no rhodopsin was detected.

Photons within the visible spectrum represent a very small fraction of the total photon flux emitted by a black body at 350 °C. But they may be sufficient to exceed the presumed threshold in shrimp[24]. If so, effective photon absorption by *R. exoculata* would be maximal at about 600 nm, given the spectral sensitivity of its rhodopsin. As a test of visibility of hot objects

with a 500 nm pigment, we demonstrated that dark-adapted humans can see a laboratory hot plate heated to 375 °C. *In situ* measurements of ambient light levels and the spectral characteristics and attenuation at hydrothermal vents are only just beginning[25].

We have demonstrated that the vent shrimp, *Rimicaris exoculata*, previously thought to be eyeless, has a thoracic eye that is well adapted for detection of very dim light. Thermal radiation from high-temperature plumes at black smoker chimneys could provide the light sensed by the shrimp. The role of the thoracic eye as a visual organ, however, will remain unresolved until more is known about its physiology and its unusual photic environment.

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Chapter 10

Light Emission at Deep-Sea Hydrothermal Vents

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Running Head: Light at hydrothermal vents

This manuscript is submitted for consideration as a *Note* in L&O.

Abstract --- A CCD camera was used to detect light emitted from the base of hot (~350°C) plumes that rise from the orifice of active sulfide chimneys on the Endeavour Segment of Juan de Fuca Ridge. Our calculations indicate that thermal radiation from hot water may account for most of the light detected and that this light may be sufficient for geothermally-driven photosynthesis by bacteria.

Low-level light emission at deep-sea hydrothermal vents was recently hypothesized as a consequence of studies on a novel visual organ in shrimp that colonize high temperature black smokers on the Mid-Atlantic Ridge (Van Dover et al., In Press; Pelli and Chamberlain, In Press). We reasoned that this hypothetical radiation could be a general phenomenon occurring at any high-temperature vent. An opportunity to image ambient light levels at vents using a CCD (Charge Couple Device) camera arose during an *Alvin* dive series at hydrothermal sites on the Endeavour Segment of the Juan de Fuca Ridge (47°57'N; 2200 m) in the northeastern Pacific Ocean (Tivey and Delaney, 1986). Photon-to-electron conversion in the camera (quantum efficiency, η) is wavelength-dependent and is maximal (40%) at 650 nm; η is >5% for 400 nm < λ < 950 nm. The camera was stably positioned 45 cm from the orifice of a black smoker chimney; water temperature 3 cm within the throat of the smoker measured 356°C. Images were collected with all external lights extinguished and with portholes blacked-out. CCD images collected under ambient light conditions show an irregular but sharply-defined line of light that follows the sulfide-plume interface, with the light extending upward in the plume, becoming unevenly dimmer and more diffuse (Fig. 1). The same phenomenon was observed at two

active chimneys 50 m apart. The light was not detectable by non-dark-adapted human eyes. The images suggest that light intensity is likely to be correlated with temperature and percentage of hydrothermal fluid. The brightest group of pixels in a series of 10 s images of the same plume consistently gave a measured electron flux of 80±7 electrons $pixel^{-1}$ s^{-1}. Electron flux detected by the camera can be related to photon flux emitted by the source *via* quantum efficiency characteristics of the camera given above.

Thermal radiation must be emitted by the hot water; thermal radiation from a 350°C black body has an emission spectrum that peaks in the far infrared. While radiation in this wavelength is neither detected by the CCD camera nor transmitted through 0.5 m of water, the thermal spectrum has a tail that reaches into the visible region, where the light can penetrate water and be collected by the camera. Since thermal emission must provide the base-level illumination detected by the camera, we can calculate its theoretical intensity as perceived by the camera. Assuming a black body spectrum for thermal emission and an absorption spectrum corresponding to pure water over the 0.45 m path length between chimney and camera, the number of electrons counted per pixel would be given by:

$$n = Apf \int_0^\infty N_\lambda \eta_\lambda e^{-0.45 a_\lambda} d\lambda$$

where A is the area of the plume contributing to a single pixel on the image (4×10^{-7} m^2), p is the transmission through the optical system of the camera (estimated at 0.5), f is the solid angle subtended by the camera lens at the object (3×10^{-4} sr), N_λ is the spectral photon

radiance of a 350°C black body at the appropriate temperature, η_λ is the quantum efficiency of the camera, and a_λ is the absorption coefficient of pure water. This calculation yields a spectral curve strongly peaked at 800-900 nm, limited at short wavelengths by the black body spectrum and at long wavelengths by the combined factors of water absorption and detector quantum efficiency. Calculated flux of electrons from an ideal black body radiator, as detected by the CCD camera, is strongly temperature-dependent. Vent water at 350°C yields 390 electrons $pixel^{-1}$ s^{-1}; 300°C water yields 40 electrons $pixel^{-1}$ s^{-1}. These theoretical calculations compare well with the observed brightest flux, suggesting that thermal radiation might provide the major component of the light observed. Thermal radiation from a plume of hot water will not be that of a perfect black body radiator, nor will absorption of photons by seawater be equal to that of pure water. Other physical and chemical sources of light may be present, such as emissions during oxidation-reduction reactions that take place when reduced vent water mixes with oxygenated seawater.

The existence of a steady source of light at depths below the euphotic zone raises the possibility of geothermally-driven photosynthesis (GDP). A photon flux at 0.0005% of sunlight ($9x10^{15}$ photons m^{-2} s^{-1}) is sufficient for photosynthesis by macroalgae (Littler et al. 1985). Bacteriochlorophyll *b* with an absorption maximum centered around 1050 nm (Thornber et al. 1978) and positioned 5 cm from a 350°C source of black body radiation (in pure water) would encounter a photon flux of about $2x10^{16}$ photons m^{-2} s^{-1}. Shifting the absorption peak further into the infrared, increasing the temperature of the water, and decreasing the distance between the source of

radiation and the pigment would increase the photon flux encountered by the pigment. Thus, energetic requirements for active GDP by organisms similar to purple photosynthetic bacteria with bacteriochlorophyll *b* may be met near high-temperature vents. A full spectral analysis of *in situ* radiation and light attenuation characteristics of seawater in the vicinity of the plumes will yield more precise estimates of photon flux at photochemically active wavelengths.

We thank the *Alvin* Group and the Master and Crew of the *R/V Atlantis II* for their support at sea. Funds for this research were provided by an Ocean Ventures Award to CLVD and grants from the National Science Foundation and Washington Sea Grant to JRD. This is WHOI contribution number ____.

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Figure Legend

Figure 1. A set of three images of the same black smoker chimney collected with a CCD camera under varying degrees of illumination. These images were spatially co-registered and processed to remove sensor gradients from the CCD camera. A) The turbulent, thermal plume emitted from the orifice of the sulfide chimney is illuminated by a Thallium Iodide lamp mounted on the sail of the submersible. Integration time for this picture was 100 msec; only the reflected illumination is detectable. B) A 10 s integration of combined emitted radiance from the plume and reflected irradiance from a flashlight which illuminated the scene for less than 0.3 s. C) A 10 s integration of only the emitted radiance from the plume. Background noise level of the camera is perceptible in images B and C since the maximum radiation detected is approximately 20 times above the noise level of the camera.

A
B
C
2CM

APPENDIX

Chapter 11

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Deep-water zooplankton of the Guaymas Basin hydrothermal vent field

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Abstract—Zooplankton from the Guaymas Basin deep-sea vent field were collected with a 1 m^2 MOCNESS to examine the distribution of total standing stock, taxonomic composition, size-frequency distribution of zooplankton, and the species composition of calanoid copepods. Low altitude (~100 m above the bottom) horizontal tows along and across the axis of the basin's southern trough, and oblique tows from the bottom of the basin (~2000 m) to the surface were made. Total biomass in near-bottom samples (range: 13–46 cc/1000 m^3) was only about a factor of 10 lower than in the upper 100 m. However, there was little or no evidence for enrichment of biomass in the ~100 m zone above the vent site relative to biomass at the same depth horizon over non-vent areas. Total numbers of individuals ranged between 2600 and 4800/1000 m^3. Calanoid copepods consistently ranked first in abundance of counts of the taxa, followed by cyclopoid copepods, ostracods, chaetognaths, and amphipods. Other less abundant taxa, but in some cases important contributors to total biomass, were coelenterates (siphonophores, medusae), decapod shrimp, and polychaetes.

Size-frequency analysis of individuals from each taxon indicated that the biomass and abundance spectra do not fit the theoretically expected spectra based on weight-dependent metabolism and growth. The pyramid of biomass was substantially different from the pyramid of numbers in this deep-sea community.

Of the 67 species of copepods identified in two samples taken on low altitude tows, only 15 co-occurred in both samples. Many of the species in this relatively diverse community remain to be described. Larval and post-larval forms of benthic clams, gastropods, polychaetes, and crustaceans associated with the vents were collected 100–200 m above the southern trough, indicating the post-larvae may play an active role in dispersal of hydrothermal vent species.

INTRODUCTION

HYDROTHERMAL vents represent the epitome of an isolated community because (a) they have an *in situ* food resource not directly linked to photosynthetic primary production, (b) they have a unique benthic species composition, and (c) distances between vent fields may be hundreds of kilometers. The decade since the first deep-sea vent site was studied has resulted in some understanding of community structure in terms of species composition, abundance, and biomass, and of the dynamics of the sessile, benthic animals and microorganisms that co-occur there (see, for example, the volume edited by JONES, 1985). Only a small amount of work, however, has focused on the benthopelagic fauna which inhabits the waters above the vents (FLEMINGER, 1983; SMITH, 1985; BERG, 1985; VAN DOVER *et al.*, 1985; BERG and VAN DOVER, 1987). There is little known about the

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biomass, abundance, size-frequency distribution, or species composition of the benthopelagic animals in water overlying vents or how these features compare with other zooplankton communities. Water column anomalies associated with hydrothermal activity in Guaymas Basin have been described (CAMPBELL and GIESKES, 1984; MEREWETHER *et al.*, 1985), and hydrothermal plumes have been tracked on the Juan de Fuca Ridge (BAKER *et al.*, 1985; BAKER and MASSOTH, 1986). Elevated measures of microbial biomass and activity of manganese-scavenging bacteria have been shown to be associated with the Juan de Fuca plumes (WINN *et al.*, 1986; COWEN *et al.*, 1986). WINN *et al.* (1986) hypothesize that bacterial biomass in plumes may be a source of organic carbon and energy for pelagic organisms.

The purpose of the sampling described herein was to characterize the zooplankton populations within 100–200 m of the sea floor in the vicinity of the Guaymas Basin hydrothermal vent activity and to compare animals caught in this region with animals caught in adjacent areas outside the immediate influence of the vents and in the water column above the vents. Of additional interest are planktotrophic larval forms, since for many vent species, the mechanisms of dispersal from one vent site to another remain unknown.

METHODS

A 1-m^2 Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) equipped with 9 nets (333 μm mesh; WIEBE *et al.*, 1976, 1985a) was used to sample the zooplankton above the Guaymas Basin deep-sea vent field (southern trough) between 26 July and 1 August 1985. Net traps and net bar stops were present on the system to minimize contamination of the samples by preventing net bar movement when the nets were open or closed. The system carried sensors to measure pressure, temperature, conductivity downwelling light, altitude above the bottom, flow past the net, and net frame angle. Data were transmitted to the ship via conducting cable where they were microcomputer processed, displayed, and stored on both 1/4 in. cassette tapes and 5 1/4 in. floppy discs.

Two towing strategies were used (Figs 1 and 2; Table 1). Four long horizontal tows at an altitude of approximately 100 m above the bottom were taken in the southern trough: three along the axis of the trough and one perpendicular to the trough. On the first tow, the flowmeter failed and estimates of the volume filtered by each net had to be computed based on a regression relationship ($P < 0.05$) between the angle of the net and the volume filtered per unit time for the other three horizontal tows. For each of these tows, a total of eight samples was collected along the horizontal portion of the tow line. Six oblique tows were made which, when taken together, bracketed the entire 2000 m water column. Three oblique tows were to 1000 m; depth strata sampled were 1000–850, 850–700, 700–550, 550–400, 400–300, 300–200, 200–100 and 100–0 m. Two oblique tows were intended to sample 1950–1800, 1800–1700, 1700–1600, 1600–1450, 1450–1300, 1300–1150, 1150–1000 and 1000–850 m, but only one was successfully completed. The other obtained samples from 1950 to 1800 and 1800 to 1700 prior to a battery failure. The last oblique tow sampled 25 m intervals from 200 m to the surface.

Samples were analysed in a variety of ways to characterize the plankton for comparison with other studies of benthopelagic plankton and of upper water column plankton. Total biomass of all samples was measured by the displacement volume method

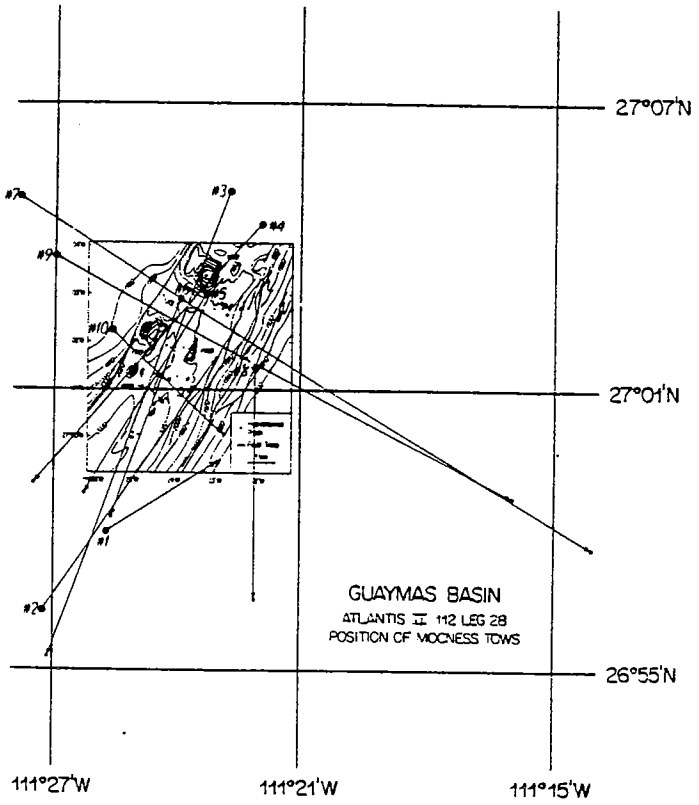

Fig. 1. Horizontal track of R.V. *Atlantis II* during MOCNESS tows taken in Guaymas Basin in July and August 1985. Direction of tow indicated by arrow. Tow numbers correspond to tow designations in Fig. 2.

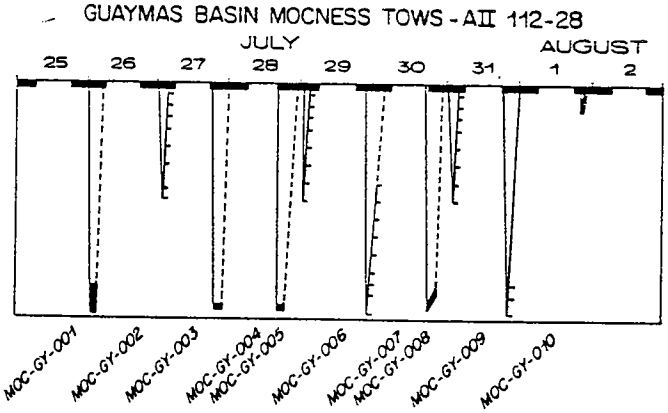

Fig. 2. Vertical extent and timing of MOCNESS tows taken in the Guaymas Basin in July and August 1985. Solid lines indicate where samples were taken; dashed lines indicate trajectory of the net system with all nets closed. Horizontal black bars indicate night-time periods.

Table 1. Guaymas Basin MOCNESS tow information

MOCNESS Tow no.	Date 1985	Local time (h)	Lat. (N)	Long. (W)	Type of tow	Number of samples
MOC-GY-01	26 July	0005	26 57.98	111 25.70	Horizontal	8
		0444	27 00.14	111 20.55	1979–1716 m	
MOC-GY-02	27 July	0106	26 56.19	111 27.22	Oblique	8
		0408	27 01.66	111 22.92	0–1000 m	
MOC-GY-03	27 July	1850	27 05.09	111 22 74	Horizontal	8
	28 July	0012	26 54.78	111 26.50	1895–1930 m	
MOC-GY-04	28 July	1820	27 04.46	111 21.91	Horizontal	8
		2327	26 59.59	111 27.00	1885–1962 m	
MOC-GY-05	29 July	0018	27 02.96	111 23.26	Oblique	8
		0318	26 58.83	111 26.04	0–1000 m	
MOC-GY-06	29 July	2157	27 02.87	111 23.71	Oblique	8
	30 July	0320	26 55.42	111 27.13	1941–850 m	
MOC-GY-07	30 July	1706	27 04.12	111 27.51	Horizontal	8
		2328	26 56.81	111 12.01	1894–1750 m	
MOC-GY-08	31 July	0100	27 03.01	111 23.73	Oblique	8
		0407	26 56.68	111 22.08	0–1000 m	
MOC-GY-09	31 July	1909	27 03.59	111 26.55	Oblique	2
	31 July	2353	26 58.87	111 16.30	1905–1700 m	
MOC-GY-10	1 August	2016	27 02.26	111 25.55	Oblique	8
		2135	26 59.99	111 22.74	0–200 m	

Local time was in the +7 time zone. First time = tow start time; second time = tow end time.

(AHLSTROM and THRAILKILL, 1963; WIEBE *et al.*, 1975) within a period of approximately 2 months after collection; *sensu stricto*, displacement volume is a biovolume measure, but here we include it as a measure of zooplankton biomass. For two tows, MOC-GY-03 and MOC-GY-07, counts were made of the major taxonomic groups present in the samples. Large individuals were sorted and counted from the entire sample; smaller organisms in a 1/4 aliquot of the sample were counted with the aid of a binocular microscope. In the process of counting taxa, larval molluscs, crustaceans, and polychaetes were sorted from the samples for identification. A silhouette photograph was made of a 1/4 aliquot of MOC-GY-03 sample 4 and MOC-GY-07 sample 7 for analysis of the size-frequency distribution of the major taxonomic groups. Size analysis was similar to that described by DAVIS and WIEBE (1985) except that all individuals on the photograph were measured; the length to biomass regressions and equipment were the same. These same samples were also used for identification and enumeration of copepod species using a binocular microscope.

The volume of water filtered by each net was used to standardize the biomass, taxonomic counts, size-frequency measurements, and species counts to a per 1000 m^3 basis. Weight classes in the size spectra are in octaves (PLATT and DENMAN, 1978).

Guaymas Basin biomass and abundance spectra were compared to data presented by RODRIGUEZ and MULLIN (1986) from the North Pacific Central Gyre (surface 0–100 m) and from Gulf Stream ring 82-B in June 1982, and to PLATT and DENMAN's (1977, 1978) theoretical spectra. Gulf Stream ring zooplankton were collected with a MOCNESS equipped with 333 μm mesh and analysed in the same manner as the Guaymas Basin samples. Two depth strata, 0–25 m (surface) and 900–1000 m (bathypelagic), from the Gulf Stream ring were used to compare surface, bathypelagic, and benthopelagic Guaymas Basin samples. [Note, we will use the WISHNER (1980a) abbreviation of "mab" for the phrase "meters above bottom".]

RESULTS

Total biomass

Biomass of the benthopelagic zooplankton collected in the horizontal tows along the axis of the trough was substantial, ranging from 13.5 to 46.3 cc/1000 m^3 (Fig. 3a,b). The cross-axis tow had less biomass on average and values ranged from 12.5 to 25.9 cc/1000 m^3 (Fig. 3c). This tow, however, was shallower than the first two and the majority of its samples were taken on the eastern flank of the trough.

In the water column above the southern Guaymas Basin, biomass was highest in the upper 100 m, with values ranging from 395 to 622 cc/1000 m^3 (Fig. 4a–c). There was subsurface minimum between 600 and 700 m, evident in all three 0–1000 m tows (range: 15.3–22.4 cc/1000 m^3), a subsurface maximum between 900 and 1000 m (range: 48.1–613.5 cc/1000 m^3), a second minimum between 1100 and 1300 m (single value: 10.3 cc/1000 m^3), and a second maximum just above the deep-sea floor (single value: 107.1 cc/1000 m^3). The shallow minimum may be the result of diel migration of species which inhabit these depths during the day and move to surface waters at night.

Comparison of the biomass just above the vent field with that in the remainder of the column reveals that the biomass at depth was about a factor of 10 lower than the maximal values found in the surface waters (Figs 3 and 4). Biomass of samples from the low altitude tows was about a factor of two higher than in the water column between 1200 and 1700 m, indicating a possible biomass enhancement effect within the benthopelagic zone. The data, however, were too variable to conclude that this trend was significant.

Taxonomic counts

Counts of the major taxonomic groups in MOC-GY-003 and MOC-GY-007 (Tables 2 and 3) living within 100–200 mab reflected the relatively high biomass for this deep-sea community. Total numbers ranged from 2593 to 4817/1000 m^3 for the 16 samples collected on the two tows. While there were, on average, slightly more individuals collected on the cross-axis tow, the difference was not significant (t-test; $P > 0.05$). Within-tow variability for total number of individuals was larger than expected by chance (Poisson expectation; $P < 0.05$), indicating patchiness on the scale of a few hundred meters. There was no evidence of any gradient of zooplankton abundance along the axis of the southern trough. The cross-axis tow, however, had high counts in the basin and lower counts toward the end of the tow on the eastern flank.

The ranks of taxon abundance were very similar from one sample to the next (Kendall's concordance coefficient W; $P < 0.001$; TATE and CLELLAND, 1957), both within each tow and between tows. Calanoid copepods were numerically dominant, generally accounting for >50% of the individuals (Figs 6 and 7). Cyclopoid and harpacticoid copepods and ostracods were usually next most abundant. There was less agreement among the taxa in their variation in abundance along a tow line. Although the concordance test, performed on ranks of abundance in each sample set for each taxa separately, gave a probability of $P = 0.05$ for GY-03 and $P < 0.05$ for GY-07, the fact that we performed multiple tests requires the table probability value to be ≤ 0.0125 for significance to be at the 0.05 level. Thus, the trend toward multispecies concordance along a tow is, at best, marginally significant, i.e. there is not strong evidence for multispecies patchiness.

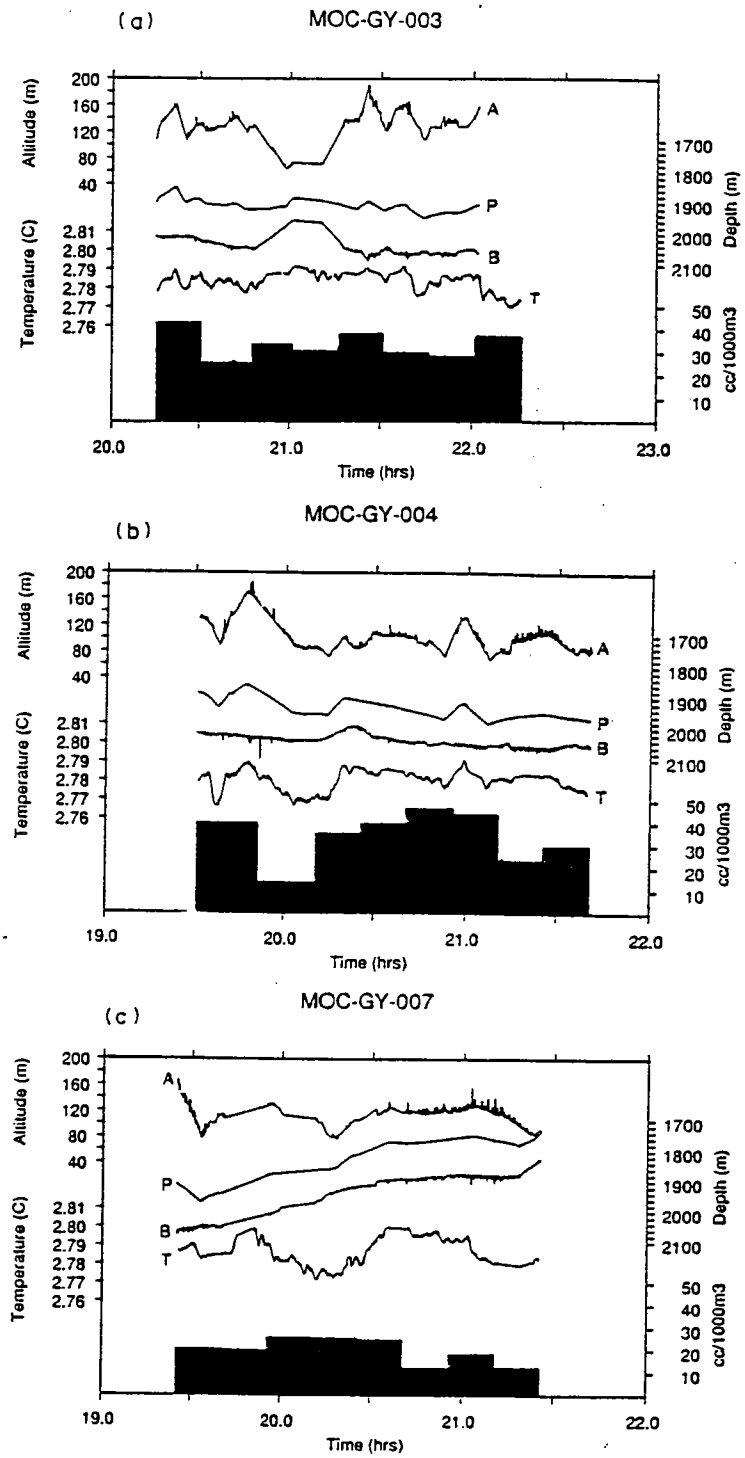

Fig. 3. Horizontal distribution of zooplankton biomass (measured as displacement volume) within 100 m of the bottom of the southern trough and flanks of the Guaymas Basin. Each sample represents a distance traveled by a net of 1000–1500 m. Plots include temperature (T), altitude (A), depth below the surface (P), and bottom depth (B) for these low altitude MOCNESS tows. (a, b) Tows parallel to the basin axis; (c) tow perpendicular to the basin axis.

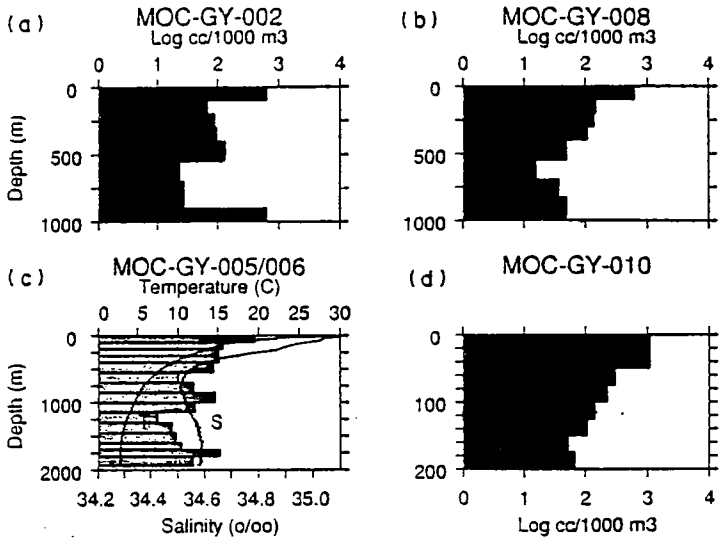

Fig. 4. Vertical distribution of zooplankton biomass in the water column above the Guaymas Basin southern trough based on five oblique MOCNESS tows. The temperature and salinity profiles in (c) were taken on MOC-GY-006.

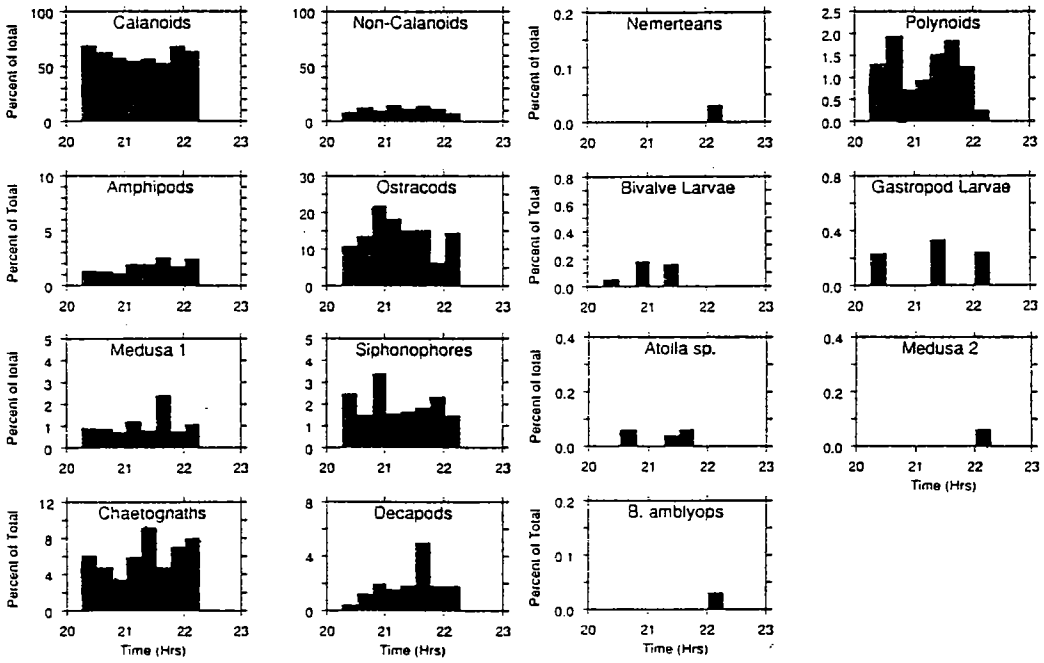

Fig. 5. Percent of total abundance for taxonomic groups on MOC-GY-003.

Table 2. Abundance (no./1000 m^3) *of taxonomic groups in MOC-GY-003*

Net no.	Mid-depth (m)	Volume filtered (m^3)	Biomass (cc per 1000 m^3)	Calanoid copepoda	Cyclopoid copepoda	Amphipoda	Ostracoda	Medusa (1)	Siphonophora	Chaetognatha
1	1896	464	43.1	3183.19	366.38	60.34	500.00	40.95	114.22	282.33
2	1911	589	25.5	1706.28	339.56	33.96	368.42	23.77	40.75	130.73
3	1902	596	33.6	2162.75	348.99	40.27	818.79	26.85	127.52	127.52
4	1895	650	30.8	2160.00	572.31	75.38	720.00	47.69	61.54	233.85
5	1910	657	38.1	2094.37	407.91	70.02	554.03	28.92	60.88	337.90
6	1930	673	29.7	1361.07	362.56	65.38	392.27	62.41	47.55	123.33
7	1924	710	28.2	2474.65	394.37	61.97	226.76	26.76	84.51	254.93
8	1920	698	35.8	3008.60	326.65	114.61	681.95	50.14	68.77	378.22
Ave.				2268.86	389.84	65.24	532.78	38.44	75.72	233.60

Net no.	Decapoda	Polychaeta larvae	Pelecypoda larvae	Gastropoda larvae	*Atolla* sp.	Clear medusa (2)	*Bentheuphausia ambylops*	Nemertea	Total
1	19.40	60.34	2.16	10.78	0.00	0.00	0.00	0.00	4648.72*
2	33.96	52.63	0.00	0.00	1.70	0.00	0.00	0.00	2731.75
3	73.83	26.85	6.71	0.00	0.00	0.00	0.00	0.00	3760.07
4	61.54	36.92	0.00	0.00	0.00	0.00	0.00	0.00	3969.23
5	68.49	54.79	6.09	12.18	1.52	0.00	0.00	0.00	3697.11
6	129.27	47.55	0.00	0.00	1.49	0.00	0.00	0.00	2592.87
7	64.79	45.07	0.00	0.00	0.00	0.00	0.00	0.00	3633.80
8	84.53	11.46	0.00	11.46	0.00	2.87	1.43	1.43	4742.12
Ave.	66.97	41.95	1.87	4.30	0.59	0.36	0.18	0.18	3721.96

*Includes fish larvae and unidentified euphausiids.

Table 3. Abundance (no./1000 m³) of taxonomic groups in MOC-GY-007

Net no.	Mid-depth (m)	Volume filtered	Biomass (cc per 1000 m³)	Calanoid copepoda	Calanoid copepoda	Amphipoda	Ostracoda	Medusa (1)	Siphonophora	Chaetognatha
1	1894	728	20.6	3038.46	494.51	89.29	384.62	21.98	54.95	170.33
2	1870	748	20.1	3000.00	443.85	80.21	561.50	28.07	74.87	155.08
3	1835	772	25.9	2150.26	1352.33	46.63	165.80	72.54	129.53	191.71
4	1786	780	25.6	2358.97	1553.85	111.54	261.54	47.44	143.59	220.51
5	1760	804	24.9	2681.59	636.82	90.80	313.43	13.68	64.68	240.05
6	1760	805	12.4	2434.78	253.42	37.27	402.48	2.48	4.97	49.69
7	1750	806	18.6	1846.15	287.84	50.87	367.25	2.48	14.89	260.55
8	1750	799	12.5	2187.73	390.49	78.85	250.31	8.76	10.01	178.97
Ave.				2462.24	676.64	73.18	338.37	24.68	62.19	183.36

Net no.	Decapoda	Polychaeta larvae	Pelecypoda larvae	Gastropoda larvae	*Atolla* sp.	Clear medusa (2)	*Bentheuphausia ambylops*	Nemertea	Total
1	65.93	65.93	27.47	32.97	0.00	0.00	1.37	0.00	4447.80
2	72.19	69.52	26.74	0.00	0.00	0.00	0.00	0.00	4512.03
3	49.22	25.91	5.18	0.00	0.00	0.00	0.00	0.00	4189.12
4	52.56	61.54	5.13	0.00	0.00	0.00	0.00	0.00	4816.67
5	110.70	29.85	14.93	4.98	1.24	1.24	1.24	0.00	4208.95*
6	64.60	19.88	4.97	0.00	0.00	0.00	0.00	1.24	3275.78
7	80.65	4.96	0.00	0.00	0.00	1.24	0.00	0.00	2916.87
8	113.89	0.00	5.01	5.01	0.00	5.01	0.00	1.25	3235.29
Ave.	76.22	34.70	11.18	5.37	0.16	0.94	0.33	0.31	3950.31

*Includes unidentified euphausiids.

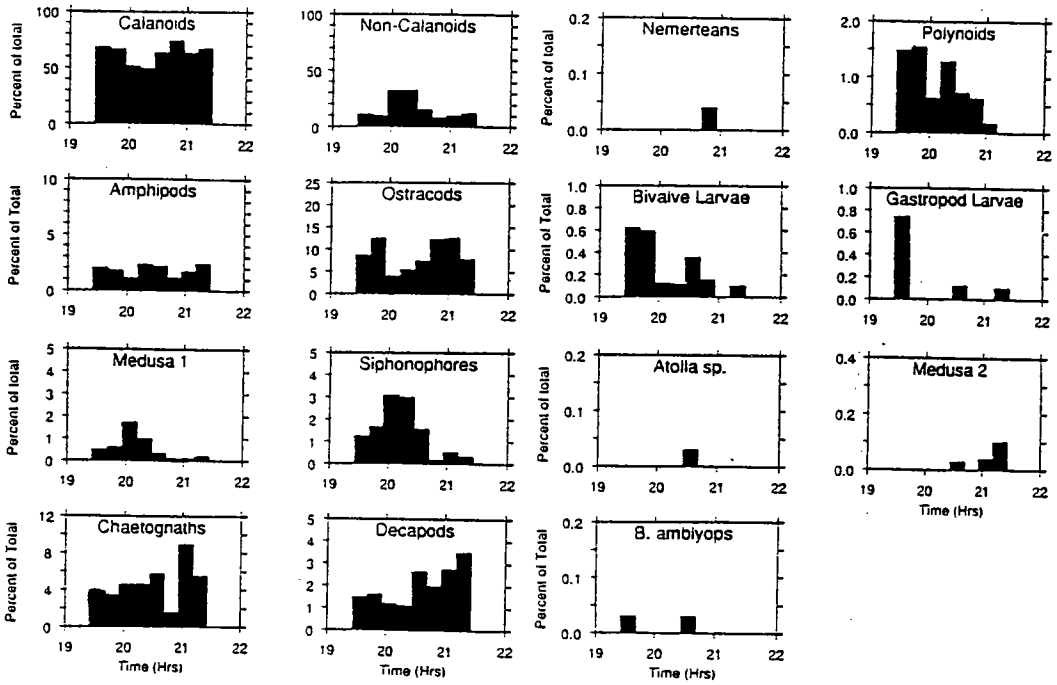

Fig. 6. Percent of total abundance for taxonomic groups on MOC-GY-007.

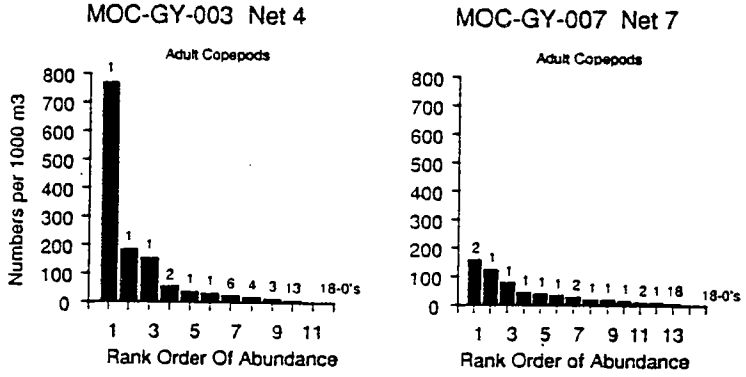

Fig. 7. Abundance of copepod species as a function of their rank order in abundance for MOC-GY-003 sample 4 and MOC-GY-007 sample 7. Numbers above each histogram indicate the number of species having that rank.

Size-frequency data

Analysis of the silhouette photographs of one sample from each of the tows on which the taxa were enumerated permits us to evaluate the contribution of the major taxa to the total biomass of the samples and provides insight into the position of the taxa in the size-frequency spectrum. Total counts from the silhouettes were in very close agreement with an independent aliquot counted with a microscope (compare Tables 2 and 3 with Tables 4 and 5). Estimates of biomass based on the summation of all individual size categories are

Table 4. Wet weight and abundance of major taxa as a function of size determined from silhouette analysis of MOC-GY-003 sample 4. Numbers in parentheses are total counts from the sample

	Cyclopoida (90)		Calanoida (401)		Chaetognatha (35)		Decapoda (5)		Ostracoda (90)	
	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)
Mean	0.91	0.08	2.15	0.94	12.80	4.28	25.91	354.05	1.80	0.66
Minimum	0.50	0.01	0.56	0.02	5.05	0.14	15.12	45.02	0.75	0.04
Maximum	1.52	0.28	6.26	14.85	16.66	9.38	41.35	1014.18	2.57	1.62
Wt class	Size-frequency distributions									
(mg)	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3
0.02	92.31	0.001	18.46	0.0003	–	–	–	–	–	–
0.03	153.85	0.01	12.31	0.0005	–	–	–	–	24.62	0.001
0.06	153.85	0.01	80.00	0.01	–	–	–	–	18.46	0.001
0.13	116.92	0.02	215.38	0.03	6.15	0.0009	–	–	49.23	0.01
0.25	36.92	0.01	369.23	0.10	–	–	–	–	104.62	0.03
0.50	–	–	443.08	0.25	–	–	–	–	116.92	0.07
1	–	–	966.15	1.05	24.62	0.03	–	–	215.38	0.22
2	–	–	307.69	0.56	30.77	0.06	–	–	24.62	0.04
4	–	–	43.08	0.18	104.62	0.48	–	–	–	–
8	–	–	6.15	0.04	49.23	0.35	–	–	–	–
16	–	–	6.15	0.09	–	–	–	–	–	–
32	–	–	–	–	–	–	6.15	0.28	–	–
64	–	–	–	–	–	–	6.15	0.38	–	–
128	–	–	–	–	–	–	–	–	–	–
256	–	–	–	–	–	–	6.15	1.24	–	–
512	–	–	–	–	–	–	6.15	2.75	–	–
1024	–	–	–	–	–	–	6.15	6.24	–	–
2048	–	–	–	–	–	–	–	–	–	–
4096	–	–	–	–	–	–	–	–	–	–
Totals	553.85	0.04	2467.69	2.32	215.38	0.92	30.77	10.89	553.85	0.36
% Comp	13.62	0.17	60.67	8.99	5.30	3.57	0.76	42.27	13.62	1.14

Table 4. Continued

	Amphipoda (3)		Medusae (6)		Siphonophora (13)		Polychaeta (18)		Net total (661)	
	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)
Mean	7.74	14.30	11.56	290.33	4.83	2.64	3.29	0.35	2.87	6.34
Minimum	2.92	0.77	6.41	31.38	2.50	1.27	1.75	0.04	0.50	0.01
Maximum	11.62	28.74	18.22	833.65	6.72	3.78	5.94	1.57	41.35	1014.18
Wt class (mg)	Size-frequency distributions									
	no./1000 m³	g/1000 m³	no./1000 m³	g/1000 m³	no./1000 m³	g/1000 m³	no./1000 m³	g/1000 m³	no./1000 m³	g/1000 m³
0.02	–	–	–	–	–	–	–	–	110.77	0.002
0.03	–	–	–	–	–	–	6.15	0.0003	196.92	0.01
0.06	–	–	–	–	–	–	12.31	0.0009	264.62	0.02
0.13	–	–	–	–	–	–	18.46	0.003	406.15	0.06
0.25	–	–	–	–	–	–	30.77	0.01	541.54	0.15
0.50	–	–	–	–	–	–	36.92	0.02	596.92	0.33
1	6.15	0.005	–	–	6.15	0.01	–	–	1218.46	1.31
2	–	–	–	–	43.08	0.10	6.15	0.01	412.31	0.76
4	–	–	–	–	30.77	0.11	–	–	178.46	0.77
8	–	–	–	–	–	–	–	–	55.38	0.39
16	6.15	0.08	–	–	–	–	–	–	12.31	0.17
32	6.15	0.18	6.15	0.19	–	–	–	–	18.46	0.65
64	–	–	6.15	0.41	–	–	–	–	12.31	0.79
128	–	–	6.15	0.65	–	–	–	–	6.15	0.65
256	–	–	6.15	1.31	–	–	–	–	12.31	2.55
512	–	–	6.15	3.03	–	–	–	–	12.31	5.78
1024	–	–	6.15	5.13	–	–	–	–	12.31	11.37
2048	–	–	–	–	–	–	–	–	–	–
4096	–	–	–	–	–	–	–	–	–	–
Totals	18.46	0.26	36.92	10.72	80.00	0.21	110.77	0.04	4067.69	25.77
% Comp.	0.45	1.02	0.91	41.59	1.97	0.82	2.72	0.15		

Table 5. Wet weight and abundance of major taxa as a function of size determined from silhouette analysis of MOC-GY-007 sample 7. Numbers in parentheses are total counts from the sample

	Cyclopoida (115)		Calanoida (380)		Chaetognatha (40)		Decapoda (12)	
	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)
Mean	1.04	0.12	2.01	0.91	12.10	4.83	13.19	44.85
Minimum	0.50	0.01	0.56	0.02	4.48	0.09	6.52	3.32
Maximum	2.14	0.73	6.98	20.15	25.08	39.26	24.79	207.90
Wt class (mg)	Size-frequency distributions							
	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3
0.02	29.78	0.001	9.93	0.0002	–	–	–	–
0.03	99.26	0.01	24.81	0.001	–	–	–	–
0.06	183.62	0.01	114.14	0.01	–	–	–	–
0.13	193.55	0.02	218.36	0.03	9.93	0.001	–	–
0.25	49.63	0.01	416.87	0.12	–	–	–	–
0.50	14.89	0.01	401.99	0.22	4.96	0.002	–	–
1	–	–	426.80	0.47	44.67	0.05	–	–
2	–	–	203.47	0.40	49.63	0.11	–	–
4	–	–	49.63	0.21	59.55	0.28	4.96	0.02
8	–	–	9.93	0.07	19.85	0.16	9.93	0.10
16	–	–	9.93	0.19	–	–	19.85	0.36
32	–	–	–	–	9.93	0.36	9.93	0.30
64	–	–	–	–	–	–	4.96	0.29
128	–	–	–	–	–	–	4.96	0.58
256	–	–	–	–	–	–	4.96	1.03
512	–	–	–	–	–	–	–	–
1024	–	–	–	–	–	–	–	–
2048	–	–	–	–	–	–	–	–
4096	–	–	–	–	–	–	–	–
Totals	570.72	0.06	1885.86	1.72	198.51	0.96	59.55	2.67
% Comp.	17.94	0.94	59.28	29.14	6.24	16.21	1.87	45.17

Table 5. Continued

	Ostracoda (87)		Amphipoda (3)		Siphonophora (14)		Net total (641)	
	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)	Length (mm)	Wet wt (mg)
Mean	1.63	0.52	6.73	12.14	8.57	4.96	2.69	1.86
Minimum	0.56	0.02	2.71	0.63	5.03	2.75	0.50	0.01
Maximum	2.69	1.86	12.10	31.93	11.37	6.74	25.08	207.90
Wt class (mg)	Size-frequency distributions							
	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3	no./1000 m^3	g/1000 m^3
0.02	24.81	0.0004	–	–	–	–	64.52	0.002
0.03	29.78	0.001	–	–	–	–	153.85	0.01
0.06	–	–	–	–	–	–	297.77	0.02
0.13	44.67	0.01	–	–	–	–	466.50	0.06
0.25	89.33	0.02	–	–	–	–	555.83	0.15
0.50	119.11	0.07	4.96	0.003	–	–	545.91	0.30
1	114.14	0.11	–	–	–	–	585.61	0.63
2	9.93	0.20	–	–	4.96	0.01	267.99	0.53
4	–	–	4.96	0.02	9.93	0.05	129.03	0.58
8	–	–	–	–	4.96	0.03	44.67	0.37
16	–	–	–	–	–	–	29.78	0.55
32	–	–	4.96	0.16	–	–	24.81	0.81
64	–	–	–	–	–	–	4.96	0.29
128	–	–	–	–	–	–	4.96	0.58
256	–	–	–	–	–	–	4.96	1.03
512	–	–	–	–	–	–	–	–
1024	–	–	–	–	–	–	–	–
2048	–	–	–	–	–	–	–	–
4096	–	–	–	–	–	–	–	–
Totals	431.76	0.23	14.89	0.18	19.85	0.10	3181.14	5.91
% Comp.	13.57	3.82	0.47	3.06	0.62	1.66		

Table 6. Percent contribution of major taxa in sample 4 (MOC-GY-003) and sample 7 (MOC-GY-007) to numbers and wet weight based on the silhouette data in Tables 4 and 5

Taxa	Percent numbers		Percent wet weight	
	Sample 4 MOC-GY-003	Sample 7 MOC-GY-007	Sample 4 MOC-GY-003	Sample 7 MOC-GY-007
Calanoida	60.67	59.28	8.99	29.14
Cyclopoida/ Harpacticoida	13.62	17.94	0.17	0.94
Ostracoda	13.62	13.57	1.41	3.82
Chaetognatha	5.30	6.24	3.57	16.21
Polychaeta	2.72	0.00	0.15	0.00
Siphonophora	1.97	0.62	0.82	1.66
Medusae	0.91	0.00	41.59	0.00
Decapoda	0.76	1.87	42.27	45.17
Amphipoda	0.45	0.47	1.02	3.06

only one half to one third of the estimates based on displacement volume. There is, however, a correction to be made for comparing wet weight to displacement volume; WIEBE *et al.* (1975) found wet weight of zooplankton was on average 72–73% of displacement volume which would make the discrepancy much smaller. In addition, we suggest that a number of the gelatinous forms, such as ctenophores, which were originally present in the samples and contributed to the displacement volumes, had disintegrated in the formalin by the time the silhouettes were made, one and half years later. Thus we conclude that silhouette analysis of the taxonomic contributions to numbers is reasonably accurate and biomass is biased principally by absence of some gelatinous forms.

Of the abundant taxa, cyclopoid and harpacticoid copepods were the smallest, with a mean length of about 1 mm, and the decapod shrimp were the largest (mean ~26 mm on MOC-GY-003; mean ~13 mm on MOC-GY-007). Rank order of size for those organisms present in both sets of samples was nearly identical, although the mean size of the individuals of most taxa on MOC-GY-007 was smaller than on MOC-GY-003. The decapods were, in spite of their low numbers, the major contributors to the total biomass estimate on both tows; they accounted for 42% on MOC-GY-003 and 45% on MOC-GY-007 (Table 6). On MOC-GY-003, medusae were also an important contributor, accounting for 42% of the biomass. Calanoid copepods contributed 29% of the biomass on MOC-GY-007 and 9% on MOC-GY-003, and cyclopoid and harpacticoid copepods contributed 1% or less. The smaller total biomass in MOC-GY-007 samples, especially at the end of the tow away from the basin axis, appears to be due largely to a reduction in the size of the decapod shrimps and the absence of medusae from these samples. Thus, in these samples, the pyramid of biomass is substantially different from the pyramid of numbers.

Species composition

Individual calanoid copepods from the 1/4 aliquots used to make the silhouette photographs were identified to species (where possible) and counted (Table 7). Sixty-five species were discriminated in quarter splits of MOC-GY-003 and MOC-GY-007, although many could not be assigned to known species (a WHOI technical report is being prepared which illustrates the undescribed species). Two males of questionable identity were also counted, bringing the total to 67. Fourteen of the species were identified from

Table 7. Guaymas Basin copepods; abundance (no./1000 m^3) *of female (F), male (M), and copepodite (C) copepods counted in MOC-GY-003 sample 4 and MOC-GY-007 sample 7. Asterisked categories are not included in total species counts*

Species	MOC-GY-003 no. 4			MOC-GY-007 no. 7		
	F	M	C	F	M	C
Sp. nr. *Aetideus* sp.	6.2	0	0	0	0	0
Sp. nr. *Amallothrix*	12.3	12.3	61.5	5	0	54.6
Augaptilidae sp. 1	0	0	0	5	0	0
A. sp. 2	0	0	0	0	0	5
A. sp. 3	0	0	0	0	0	5
A. sp. 4	0	0	6.2	0	0	0
Calanus pacificus	0	0	18.5	0	0	9.9
Candacia magna	36.9	18.5	153.8	44.7	79.4	59.6
Centropages furcatus	0	0	0	5	0	0
Cephalophanes sp.	6.2	0	0	0	0	0
Chiridius nr. *gracilis*	184.6	0	104.6	5	0	5
Clausocalanus arcuicornis	6.2	0	0	0	0	0
C. furcatus	24.6	0	0	5	0	0
Eucalanus sp.	0	0	0	0	0	5
E. nr. *monachus*	12.3	0	0	0	0	0
Euchaeta sp. 1	6.2	0	0	5	0	0
E. sp. 2	6.2	0	0	0	0	0
E. sp. 3	0	0	0	9.9	0	0
**Euchaeta* spp. copepodites	–	–	221.5	–	–	273
Farrania oblongata	0	0	0	5	0	0
Gaetanus sp. (copepodites)	–	–	12.3	–	–	0
Gaidius minutus	769.2	0	307.7	158.8	0	134
G. sp. male	–	0	–	–	5	–
Sp. nr. *Gaidius*	6.2	0	0	0	0	0
Heterorhabdus nr. *abysallis*	0	0	–	9.9	19.9	–
H. nr. *compactus*	6.2	12.3	–	0	0	–
H. nr. *papilliger*	6.2	0	–	0	0	–
**H.* spp. copepodites	–	–	12.3	–	–	34.7
H. sp. female	0	–	–	5	–	–
Heterostylites longicornis	12.3	6.2	6.2	34.7	9.9	14.9
Lucicutia bicornuta	0	0	0	5	0	0
L. sp. 1	18.5	18.5	–	0	0	–
L. sp. 2 female	6.2	–	–	0	–	–
L. sp. male	–	0	–	–	5	–
**L.* spp. copepodites	–	–	6.2	–	–	0
Sp. nr. *Megacalanus*	0	0	6.2	0	0	0
Mesorhabdus sp.	0	0	0	0	5	0
Metridia macrura	0	0	0	5	0	0
M. sp. (small)	24.6	0	73.8	19.9	0	397
M. sp. (large)	12.3	0	12.3	24.8	5	19.9
Monacilla tenera	0	0	0	5	0	0
Nannocalanus minor	24.6	0	6.2	5	5	0
Phyllopus sp.	0	0	0	0	0	5
Pleuromamma gracilis	6.2	12.3	12.3	0	0	0
Rhincalanus nasutus	18.5	0	12.3	0	0	5
Scaphocalanus nr. *longifurcus*	6.2	0	0	34.7	5	0
S. male sp. 1	–	0	–	–	5	–
S. male sp. 2	–	0	–	–	5	–
Scolecithricella nr. *emarginata*	6.2	0	0	0	0	0
S. nr. *marquesa*	6.2	0	0	0	0	0
Scolecithrix danae	0	0	6.2	0	0	0
Sp. nr. *Scottocalanus*	0	0	0	0	0	5
Spinocalanus sp. 1	153.8	0	49.2	158.8	0	193.5
S. sp. 2	0	0	0	79.4	0	9.9
S. sp. 3	6.2	0	0	0	0	0
Temora discaudata	0	0	6.2	0	0	0

Table 7. Continued

Species	MOC-GY-003 no. 4 F	M	C	MOC-GY-007 no. 7 F	M	C
Temorites brevis	0	0	0	9.9	9.9	0
Temorites sp. 1	12.3	18.5	0	5	0	0
Undinella frontalis?	12.3	12.3	0	34.7	0	0
Undinella? sp. 1	24.6	0	6.2	0	0	0
Valdiviella sp.	0	0	0	0	5	0
Xanthocalanus nr. pinguis	12.3	0	0	0	0	0
Sp. A	18.5	36.9	12.3	0	0	0
Sp. B (stage V copepodite)	–	–	0	–	–	5
Sp. C	6.2	0	0	5	5	0
Sp. D	0	0	0	5	9.9	0
Other incerta sedis adults (3 spp. F; 1 sp. M)	18.5	6.2	6.2	0	0	0
*Misc. copepodites	–	–	68.2	–	–	95
No. Copepods/category	1496	154	1107.9	690.2	179	1336
Total copepods		2757.9			2205.2	
*Total no. spp. = ~67		43			41	
Total no. spp. categories = 103						

copepodites only. Seventeen species (including copepodites) co-occurred in the two samples and these were generally the most abundant copepods found in both tows, although they did not necessarily have the same relative proportions (e.g. *Chiridus* sp. 1, Table 7). Most species were found in low numbers, as indicated by the long tail on the rank order of abundance plots (Fig. 7). The percent similarity of adult copepod composition between sample 3 and sample 7 was only 46%, a value sufficiently low to be considered significantly different on the basis of criteria given by VENRICK (1982). This is also indicated by the shape of the rank order of abundance curves. Using abundances of species categories given in Table 7 combined into species groups, diversity, as indicated by the information measure of diversity, H′ (PIELOU, 1969), was 3.48 for MOC-GY-003 and 3.74 for MOC-GY-004. The equitability of numbers per species was 0.64 and 0.69, respectively. These values and the fact that many of the species found did not co-occur in the two samples indicate diversity in this benthopelagic community is relatively high.

Copepod exoskeletons were present in the samples, with abundances equal to 31% of living copepods in MOC-GY-007 and 10% in MOC-GY-003; they never reached the dominance (exoskeleton to living copepods ratios from 1.6 to 7.3) reported by WISHNER (1980b). Almost all of the exoskeletons contained some body tissue, which indicates to us they were not molts. These individuals were identified whenever possible and included in the species counts because they simply may have been individuals damaged in the nets.

A few of the species present at depth in the Guaymas Basin are known as epi- or mesopelagic forms (e.g. *Nannocalanus minor* and *Rhincalanus nasutus*). It is significant that the latter appeared consistently in both sets of samples. Although it is possible that they are contaminants that entered the nets as the MOCNESS was being set and retrieved, we believe that for the more abundant forms this is an unlikely explanation. Many other species which live in the surface waters in as great or greater abundance (CHEN, 1986; BRINTON *et al.*, 1986) were not found in the samples, and on the deep 1900+ m oblique tow that suffered battery failure, essentially no animals were present in the cod-end buckets of unopened nets. Also, as noted in the methods, the MOCNESS

was equipped with net bar traps and stops to prevent contamination through the bars. Instead, it appears that these species were present as expatriates from their typical habitat.

Two calanoid copepod species, *R. nasutus* and *Candacia magna*, were counted in all of the samples in which the taxa were enumerated (Fig. 8). Their abundance changes along tow paths were larger than expected by chance (index of dispersion; $P < 0.05$), but their fluctuations in abundance were not significantly correlated. Several larger and rarer species were also identified in these two tows. An unidentified medusa (no. 1), approximately 12 mm in diameter and with brown pigmentation, was frequently present in the deep tows along the bottom of the trough (Fig. 8). Its distribution, while patchy, showed

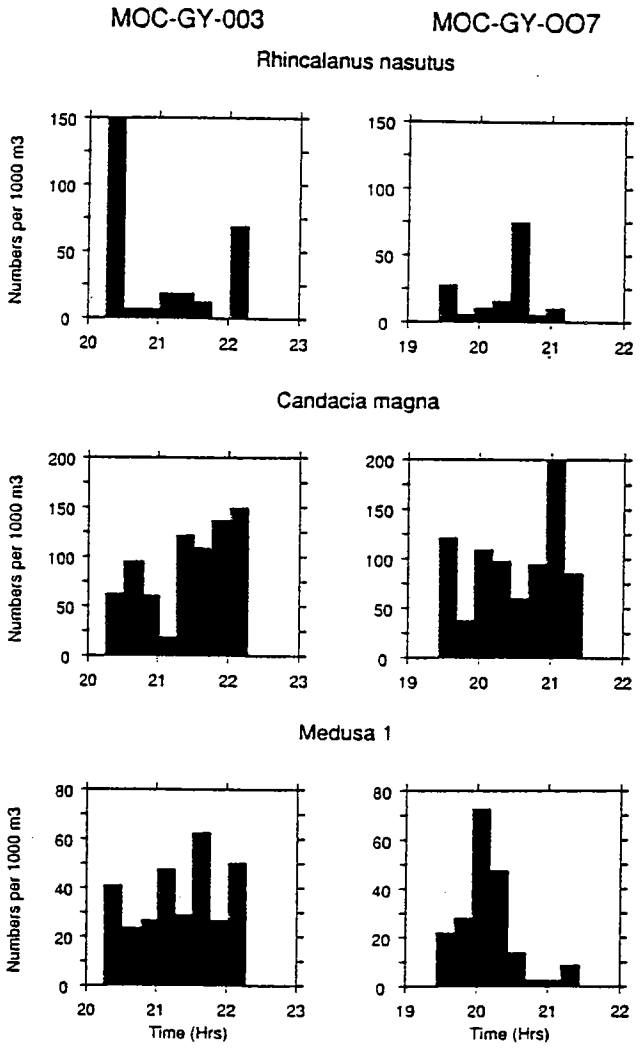

Fig. 8. Abundance of two copepods and a brown pigmented medusa (no. 1) in samples collected on MOC-GY-003 and MOC-GY-007.

no major abundance trends along the axis of the trough. In contrast, the cross-axis tow had large numbers of this medusa at the beginning and lower numbers away from the vent activity on the eastern flank of the trough. Three large specimens of the euphausiid, *Bentheuphausia ambylops*, four specimens of the medusa, *Atolla* sp., eight specimens of a clear medusa (no. 2), and three specimens of a nemertean were collected on the tows. Except for the clear medusa which usually were aggregated, the large organisms occurred sporadically.

Larval and post-larval stages of benthic invertebrates were present in all our low altitude MOCNESS tows in the Guaymas Basin. Juvenile polychaetes were most abundant and ubiquitous, with estimated densities as high as 70 individuals/1000 m^2 (Tables 2 and 3). These juveniles (length 2–6 mm) have been identified tentatively as belonging to the family Polynoidae (H. DEAN and M. PETTIBONE, personal communication). Polynoids are a typical component of the benthic macrofaunal community at hydrothermal vents and at least six species are found at Guaymas Basin vents (J. F. GRASSLE, personal communication). Bivalve and gastropod larvae and post-larvae (lengths 0.5–1 m) were widespread but less abundant (Tables 2 and 3), probably because of escape through the 333 μm mesh. Of particular significance are two types of post-larval bivalves that resemble *Calyptogena* and *Thyasira* (R. TURNER, personal communication), adults of which occur at Guaymas Basin vents. Some of the gastropod post-larvae appear to be closely allied to juveniles of gastropod species known from hydrothermal vent sites on the East Pacific Rise (TURNER *et al.*, 1985). Collection of post-larvae at altitudes of ~100 mab suggests that post-metamorphic stages may have some role in dispersal of these species. Several specimens of zoeal stages of anomuran decapods were sorted from the low altitude tows. These larvae are relatively large and appear to be lecithotrophic, with non-functional mouthparts. Both lithodid (*Neolithodes diomedeae*) and galatheid (*Munidopsis* sp.) crabs produce large eggs and undergo lecithotrophic development; adults of these species are very abundant at the Guaymas Basin vents. Anomuran larvae collected in the MOCNESS tows may eventually be identified as belonging to one of these species. A larval inarticulate brachiopod (diameter ~0.75 mm) was sorted from sample 5 MOC-GY-007; adult brachiopods have been collected from hydrothermal mounds in the Guaymas Basin (GRASSLE, 1986).

Temperature structure in the Guaymas Basin

Vertical profiles of temperature and salinity (Fig. 4) are very similar to those published by CAMPBELL and GIESKES (1984). There was a shallow (~20 m), warm (~27°C) surface layer (salinity ~35.1‰) below which a very strong thermocline (delta t = 13°C) extended from ~40 to ~200 m. Below 200 m there was a more gradual decline in temperature and salinity. A salinity minimum (34.51‰) occurred at ~750 m. This zone also has a pronounced oxygen minimum (CAMPBELL and GIESKES, 1984). Below 1500 m, temperature and salinity were nearly constant to the basin floor.

Small, hundredths of a degree changes in temperature along the low altitude tows provide evidence for hydrothermal activity in the southern basin (Fig. 3). We have used the pressure transducer depth below the surface data summed with the net transponder altitude above the bottom data to estimate the bottom depth (Fig. 3). These data show that along-axis tows MOC-GY-003 and MOC-GY-004 passed over the mound in the southern basin known as West Hill. MOC-GY-003 appears to have passed over the top (minimum altitude: 64 m); MOC-GY-004 probably passed along its eastern edge (mini-

mum altitude: 73 mm). On both of these tows, slightly lower temperature water was encountered north of the mound and patches of warmer water were encountered above and to the south for about 1000–2000 m before decreasing. Although it appears that variability in the temperature data also varies along the tow lines, this may only be an artifact introduced by the 12-bit resolution of the digitized temperature data. (The Sea Bird temperature probe is capable of resolving 0.001°C changes in temperature, but because our temperature range is set for 30°C, the digitizer limits the resolution to ~0.007°C. Thus, if the probe temperature is varying at the mid-point of the digitizing bin, no variation will be observed; if the same variation occurs near the boundary of a bin, alternation between temperature bins will give the appearance of increased variation.) The 19-point running average we used reduces this problem to some extent, but not entirely.

The cross-axis tow (MOC-GY-007, Fig. 3) shows a larger scale of temperature variation: warmest water was at the basin axis; a lower temperature patch occurred midway up the eastern flank; a second warm patch occurred near the end of the tow. During this tow, the net system stayed about 100 mab. Thus, these data could be the result of the net cutting through alternating horizontal layers of warm and cool water several 10's of meters thick, the first occurring between 80 and 150 m above the basin floor. However, a plot of the potential density anomaly vs depth (Fig. 9) reveals that the water along the tow path has very little vertical stability. In fact, the cooler water appears to be slightly more dense than the warmer water it overlies. An alternate explanation, that we sampled plumes of rising warmer water and sinking cooler water, fits the more complex picture of mixtures of lighter vent water and heavier water entering the basin from outside as described by CAMPBELL and GIESKES (1984). We cannot determine the horizontal extent of these layers or plumes from this data set.

There is no evidence that variations in biomass, taxa abundance, or species counts are correlated with the very small changes in temperature observed along the low altitude tows.

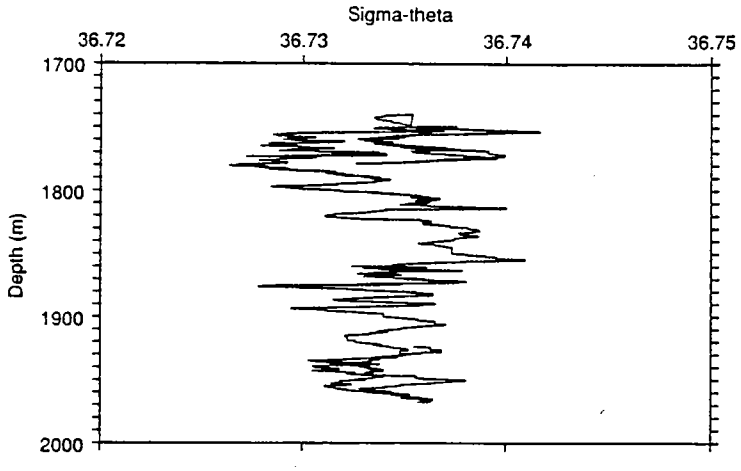

Fig. 9. Density anomaly relative to the 2000 m isobaric surface vs depth for data collected along MOC-GY-007, the tow taken perpendicular to the axis of the Southern Trough (see Fig. 3 for the tow trajectory).

DISCUSSION

We will focus on three aspects of zooplankton community structure in the Guaymas Basin: standing stock or biomass, size-frequency distribution of biomass and numbers, and taxonomic composition.

Biomass

Zooplankton biomass in Guaymas Basin MOCNESS tows decreased with depth, from a maximum of 1000 cc/1000 m^3 at the surface to <50 cc/1000 m^3 ~100 mab (total depth = 2000 m). Biomass decreased by about a factor of 10 from the surface to about 1200 m; beyond this depth, biomass remained relatively constant (Figs 4 and 10a). Concurrent sampling west of Baja California (total depth ~3600 m) with a 1 m^2 MOCNESS (HAURY *et al.*, 1986) revealed a similar distribution of biomass to a depth of 1000 m (Fig. 10b). MOCNESS tows taken in Panama Basin (bottom depth range: 3300–3875 m) in August 1975 (BISHOP *et al.*, 1986; MARRA *et al.*, 1987) again show a similar distribution of biomass to a depth of 2000 m (Fig. 10c). Of particular importance is the fact that at 2000 m in the Panama Basin, a depth well above the deep-sea floor in this area, biomass levels were nearly the same as those found at the same depth in the Guaymas Basin. This is despite the fact that there are no auxiliary primary production sources like that present in the Guaymas Basin. Zooplankton biomass/depth profiles in Guaymas Basin are also quite similar to those observed in North Atlantic Slope Water (ALLISON and WISHNER, 1986; WIEBE *et al.*, 1985b; ORTNER *et al.*, 1978). In contrast,

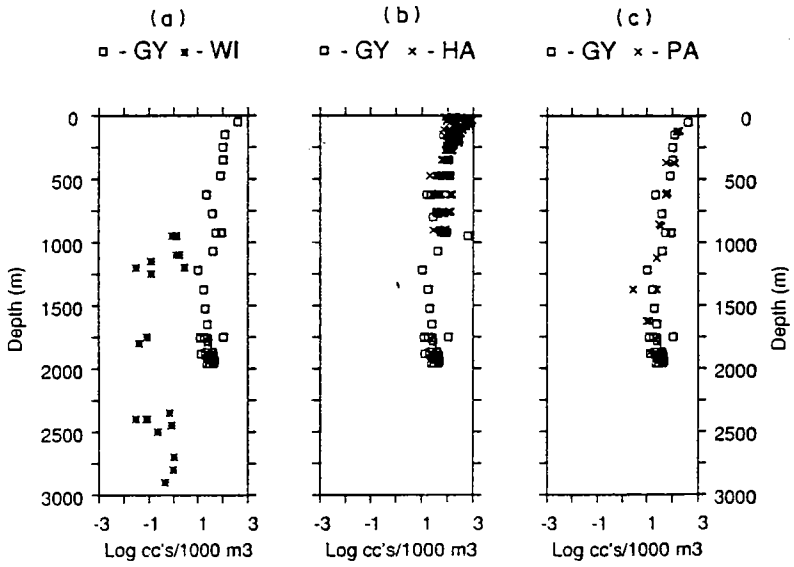

Fig. 10. Comparison of the vertical distribution of biomass of zooplankton in the Guaymas Basin with data from several other oceanographic regions. (a) Data from Guaymas Basin profiles compared with data from WISHNER (1980a). (b) Data from Guaymas Basin compared with Baja California data (HAURY *et al.*, 1986). (c) Data from Guaymas Basin compared with the Panama Basin data (MARRA *et al.*, 1987). WI, Wishner; Gy, Guaymas Basin; HA, Haury; PA, Panama Basin.

Guaymas Basin zooplankton biomass 100 mab (=Wishner's "benthopelagic" zooplankton) is more than an order of magnitude greater than that predicted by WISHNER (1980a) for depths of 1000–2000 m. Thus below 1200 m in Guaymas Basin, biomass levels sharply deviate from that expected for most open ocean sites according to VINOGRADOV (1968) and WISHNER (1980a), but are consistent with biomass patterns cited above.

For water depths of 2000 m, WISHNER (1980a) predicted benthopelagic biomass to be ~0.8% of surface biomass. Based on an *Alvin* plankton tow 100 mab, BERG and VAN DOVER (1987) report a value for benthopelagic zooplankton biomass of 0.1 g/1000 m^3 in Guaymas Basin, less than Wishner's predicted value of 1.2 for similar depths and approximately 250 times lower than what we observed for the same area. Benthopelagic biomass in the Berg and Van Dover study was 0.02–0.08% of surface biomass (depending on day or night estimates of surface biomass). The reasons why the Berg and Van Dover biomass values are so much lower than ours are not certain. Nets attached to *Alvin* are unlikely to catch larger animals as effectively as MOCNESS and since large zooplankton size classes contribute very significantly to the total biomass at depth in the Guaymas Basin, differences in net efficiency and tow speed (0.5 kn for *Alvin* vs 1.5–2.5 kn for MOCNESS) may provide the explanation.

We saw little or no evidence of a uniformly enriched zooplankton biomass immediately above the sea floor (100 mab). WISHNER (1980a) argued that the near-bottom region of the deep sea appears to be richer in organic material than the water column several hundred meters higher and suggests (based on a 3-sample comparison) that significant enrichment in zooplankton at 10 mab is a consequence. Our sampling in the Guaymas Basin does not allow us to resolve this scale of biomass distribution.

Localized enrichment of zooplankton biomass and abundance centered around chemoautotrophic production at hydrothermal vent sites on the East Pacific Rise and in Guaymas Basin have been reported by SMITH (1985) and BERG and VAN DOVER (1987). These authors find an order of magnitude enrichment in biomass, comparing vent with non-vent plankton samples collected 1–10 mab by *Alvin*. In our study, zooplankton biomass in horizontal low altitude tows (~100 mab) varied by at most a factor of 3, but the variations were not correlated to the millidegree changes in temperature which might be indicators of hydrothermal plume activity. The horizontal distance of the samples along a tow line (~750 m), however, may have resulted in the integration of much of the variability due to plume activity.

Data from WISHNER's (1980a) Tables 1 and 2 for 1000–3000 m (plotted on Fig. 10a), are one to two orders of magnitude lower than we have observed at comparable depths. The lowest of these values is from the Red Sea and has been corroborated by WEIKERT (1982). Below 1000 m, the Red Sea appears to be a unique environment with minimum temperatures of about 20°C, very little food input and no truly bathypelagic inhabitants. The only other data given by WISHNER (1980a) that falls within the depths we sampled were collected in the San Diego Trough at 10 and 100 mab. These values are about a factor of 10 lower than those obtained by HAURY *et al.* (1986) at nearby stations which, as noted above, are comparable to the Guaymas Basin data for the upper 1000 m. Several factors may account for the discrepancies between Wishner's data and data from MOCNESS samples. WISHNER (1980a) collected her samples with an opening–closing net system carrying 3 nets of 0.183 μm mesh attached to the bottom of the Deep-Tow instrument. Without net bar traps to keep the bars down after being released to an open position, we have observed that the net bars on MOCNESS can slide back up, reducing

the mouth opening of the net. Nets with mesh area to mouth opening ratios of about 4.0, like the ones Wishner used, are unlikely to accept 100% of the water presented to them (SMITH *et al.*, 1968). Deep-Tow was "flown" within 10–100 m of the deep-sea floor on long tracklines (tens of kilometers) during which time nets were opened and closed. Abrasion and destruction of animals in the cod-ends of nets is a serious problem, especially on long tows. Removal of large individuals prior to biomass analysis (WISHNER, 1980a) can lead to significant underestimates of the standing stocks of zooplankton. This underestimate should increase with depth since zooplankton tend to increase in size with depth. We suggest that the cumulative effect of these various factors could amount to between a 50 and 75% underestimate of the biomass present in the Deep-Tow tows. BERG and VAN DOVER (1987) used a net system similar to Wishner's, but attached to *Alvin*; it is likely that some of these possible errors affected their results as well.

Biomass and abundance spectra

Theoretical foundations for studies of the abundance of plankton as a function of their size are the focus of several papers (KERR, 1974; PLATT and DENMAN, 1977, 1978; SILVERT and PLATT, 1978, 1980; PLATT, 1985). These works have emphasized the importance of size or biomass spectra in understanding biomass or energy flow in a community. Platt and Denman's derivation is based on functions of weight-dependent metabolism and growth and applies to systems in steady state. Approximations of secondary production can, in principal, be calculated based on size spectra, temperature, and phytoplankton concentration (HUNTLEY and BOYD, 1984). Knowledge of the biomass in a few size classes on one end of the spectrum have been used to predict with rough accuracy the biomass in other size classes. For example, phytoplankton or zooplankton biomass has been used to predict fish biomass (SHELDON *et al.*, 1977; BORGMANN, 1982; SPRULES *et al.*, 1983; MOLONEY and FIELD, 1985), and vice versa (SHELDON *et al.*, 1977). SPRULES and MUNAWAR (1986) have used the biomass spectrum to indicate system productivity or perturbation from the steady state of several lakes in Canada when compared with oligotrophic oceanic gyres (RODRIGUEZ and MULLIN, 1986).

Spectra of biomass and abundance (Fig. 11a, b) for zooplankton in the Guaymas Basin and Gulf Stream ring are multimodal. In addition, the biomass and abundance spectra for benthopelagic Guaymas Basin zooplankton and bathypelagic Gulf Stream ring zooplankton (900–1000 m) are nearly identical. This might imply that available food resources and ecological processes are similar. The higher values of the Gulf Stream ring surface layer spectra are indicative of the greater food resources (DAVIS and WIEBE, 1985). Biomass peaks seen in Guaymas zooplankton populations are associated with specific taxonomic groups. The peak at 1 mg wet weight is predominantly calanoid copepods and ostracods, while the peak beyond 100 mg is predominantly decapods and medusae. The same groups are associated with the biomass peaks in the Gulf Stream ring at 900–1000 m, although medusae and ctenophores are more important than decapods at >100 mg. These zooplankton taxa were also responsible for peaks observed in the abundance spectra. Observations of regular, multimodal biomass peaks in benthic communities over a wide range of sampling sites and size categories have corresponded to bacteria, interstitial meiofauna, and macrofauna (SCHWINGHAMER, 1981). Our data indicate that predictable multimodal patterns might also occur in the oceanic zooplankton.

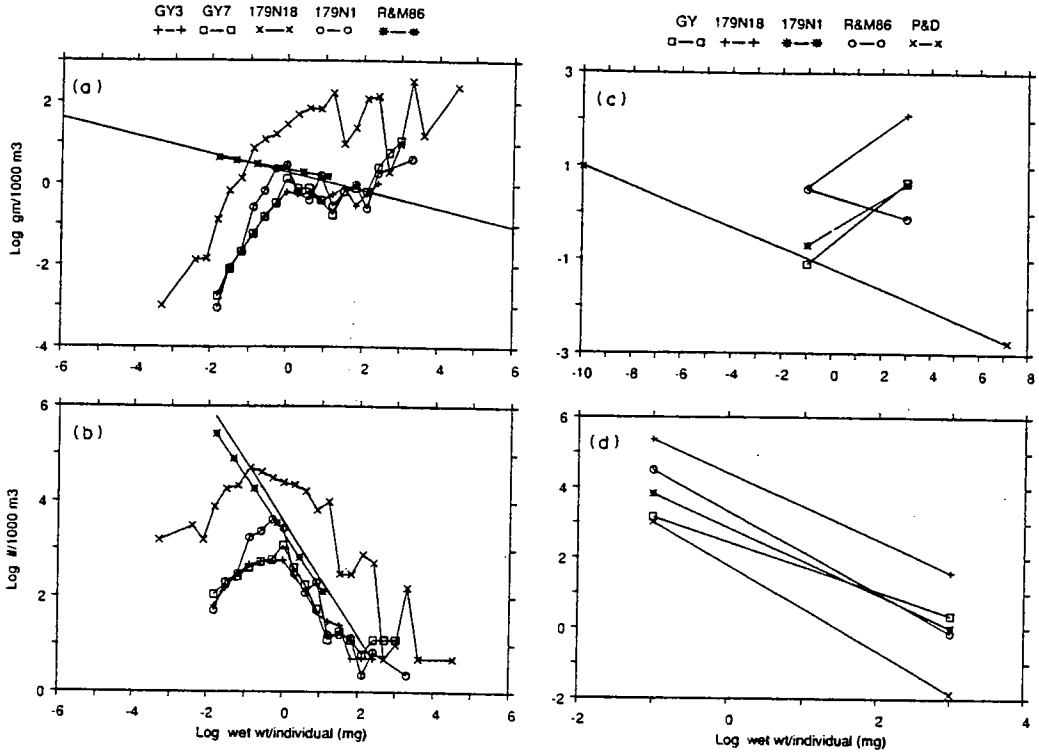

Fig. 11. (a, b) Biomass and abundance spectra for the Guaymas Basin compared with spectra from the 900–1000 m and 0–25 m strata of Gulf Stream warm-core ring 82-B and from the North Pacific Central Water Mass (from RODRIQUEZ and MULLIN, 1986). The unmarked solid line in both plots is the PLATT and DENMAN (1978) theoretical line. (c, d) Regression lines for the Guaymas Basin spectra compared with those from the warm-core ring, the North Pacific, and the theoretical expected line of PLATT and DENMAN (1978).

Regression lines for the biomass and abundance spectra (Fig. 11c, d) do not fit theoretical expectations. Data points below 0.1 mg wet weight were excluded from the regression computation to avoid possible bias resulting from escapement of individuals through the net mesh. Consistently positive biomass slopes of Guaymas Basin (0.44) and 82-B zooplankton (0.33–0.39) contrast with Pacific Ocean gyre zooplankton (–0.16) and the theoretical biomass slope (–0.22) (Fig. 11c).

Regression lines for abundance spectra are all negative (Fig. 11d). But the slope of the Guaymas Basin (–0.70) is less negative than the Pacific Ocean gyre (–1.15) and the theoretical slope (–1.22). The Gulf Stream ring slopes are also less negative (–0.92 to –0.95). Departure from the theoretically expected slope of –1.22, based on weight-dependent metabolism and growth for steady-state conditions (PLATT and DENMAN, 1977, 1978), implies either that non-steady-state conditions are operating within the zooplankton communities of the Guaymas Basin benthopelagic domain and Gulf Stream ring 82-B or that the weight-specific growth and respiration models used by Platt and Denman do not adequately account for the processes occurring in these areas. DAVIS and WIEBE (1985) argued that higher biomass and numbers seen in larger size classes in the Atlantic ring relative to expected values were indicative of an enriched, previously

oligotrophic ecosystem, which at the time of sampling was predator-dominated and not in steady state. This is not a likely case for the samples collected between 1850 and 2000 m in Guaymas Basin. This latter system should be far more stable as a result of a damped seasonal cycle of energy input from surface production and, as far as is known, approximately continuous production from hydrothermal vent activity; under such circumstances, large temporal variations in the size spectra would not be expected. Additional sampling of this deep-sea environment is required to resolve this issue.

Comparison of taxonomic composition

There is very little evidence that our samples were strongly affected by contamination with animals living in parts of the water column through which the nets passed when they were closed. Although some of the enumerated species of calanoid copepods are supposed to be near-surface dwellers, the evidence presented above suggests that they were caught at depth. This differs from interpretations of BERG and VAN DOVER (1987), who considered taxa such as the chaetognaths as epipelagic net-contaminants. If a major fraction of the chaetognath species turns out to have an epipelagic origin, then their presence at depth may indicate an important link between surface production and benthopelagic consumption.

Zooplankton community structure in the deep sea is of two distinct types. One type is the essentially monospecific culture of copepods that has been reported from a hydrothermal vent site on the East Pacific Rise (FLEMINGER, 1983; SMITH, 1985). The second type is dominated by species-rich assemblages of copepods, as reported, for example, by WISHNER (1980b), SMITH (1982), BERG and VAN DOVER (1987), and this study.

There are strong similarities between the taxonomic composition of benthopelagic zooplankton collected at various sites in the eastern Pacific and North Atlantic (WISHNER, 1980b) and our work in Guaymas Basin. Copepods comprise 50% or more of the total abundance, with ostracods and chaetognaths also relatively abundant. Wishner's samples also included a small, but significant number of isopods; this taxon was unimportant in our samples from Guaymas Basin.

WISHNER (1980b) identified over 100 species of calanoid copepods in her samples and found that a few species were numerically dominant at each site. We have identified ~67 copepod species and have found a few dominant species present. Sample size and number of habitats sampled are not identical between these two studies. Nevertheless, it is clear that diversity of deep-sea copepods can be quite high. In both this study and WISHNER's (1980b) work, there is, within the copepod populations, a high proportion of females and copepodites. In fact, the majority of species in our Guaymas Basin samples are represented only by females and/or copepodite stages. This, however, is not unusual; males of most copepod species are rare.

In plankton samples collected immediately above other hydrothermal vent communities, lysianassid amphipods comprised a significant proportion of the total abundance (SMITH, 1985; BERG and VAN DOVER, 1987) and were typically the dominant component in terms of biomass (Berg and Van Dover samples from vent sites at 21 N). Lysiannassid amphipods are more properly considered as suprabenthos rather than true zooplankton, in as much as they are closely linked with the benthic community at hydrothermal vents. Suprabenthic animals were rare in the Guaymas Basin MOCNESS samples.

BERG and VAN DOVER (1987) also report collecting a significant number of siphonostome and poecilostome copepods in addition to calanoids, harpacticoids, and cyclopoids.

Many new species of siphonostome and poecilostome copepods have been collected from hydrothermal vent areas, including Guaymas Basin (A. HUMES, personal communication). We did not collect representatives of these taxa in our tows ~100 mab over the vent field either because their small size caused them to escape capture through the mesh or because they live closer to the bottom than we generally sampled.

BERG and VAN DOVER (1987) emphasized the significance of the presence of larval stages of vent invertebrates in their plankton tows, and they note that larval stages of non-vent invertebrates were absent in their samples. The paucity or complete absence of larval stages in benthopelagic plankton tows over non-vent terrain was observed by WISHNER (1980b). Similarly, we found no specimens that are clearly the larval stages of non-vent benthic invertebrates. We did find post-larvae of vent invertebrates at 100 mab indicating the potential role of post-metamorphic stages in dispersal. Much preserved zooplankton material from Guaymas Basin MOCNESS tows remains to be examined, however, and non-vent larvae may be present in it.

CONCLUSIONS

Biomass of zooplankton within 100–200 m of the bottom of the Southern Trough of the Guaymas Basin in July/August 1985 was approximately a factor of 10 lower than the biomass in the upper 100 m of the water column. Two subsurface biomass minima occurred, one about 700 m where salinity and oxygen minima also occurred and the other about 1300 m above the Guaymas Basin sill depth.

Low altitude (~100 mab) MOCNESS tows over Guaymas Basin hydrothermal fields provide little or no evidence for elevated biomass associated with hydrothermal activity. We cannot, however, eliminate the possibility that enhanced production in hydrothermal plumes is on a spatial scale smaller than our sampling design could detect and closer to the bottom.

Calanoid copepods were the numerically dominant taxonomic group present in the low altitude MOCNESS tow samples; they comprised about 60% of the individuals in the collections. Cyclopoid and harpacticoid copepods, ostracods, and chaetognaths were next most important numerically. Along the two low altitude tows (~100 mab) in which taxa were counted, there was high concordance of rank order of abundance of taxa from one sample to the next and between samples from the two tows. The taxa showed much less concordance in their spatial patterns of abundance.

Size-frequency analysis of sample 4 from MOC-GY-003 and sample 7 from MOC-GY-007 provided biomass and abundance spectra. These spectra were very similar between the two tows and were used to provide estimates of the contribution of each of the taxa to total biomass. Large but relatively rare individuals of particular taxa (i.e. decapods, medusae) accounted for a much larger fraction of the biomass than their numbers would portend. In the Guaymas Basin deep-sea community, the pyramid of biomass does not closely follow the pyramid of numbers. None of our size-frequency spectra fit the theoretically expected spectra based on weight-dependent metabolism and growth.

Counts of the calanoid copepods in these two samples yielded 67 species, of which 15 co-occurred in both samples. Over 50% of the species could not be specifically identified and probably remain to be described. A percent similarity index of 46% indicated the species composition in sample 3 from the along-axis tow and sample 7 from the

cross-axis tow was significantly different. Species diversity and equitability of individuals among the species were relatively high.

Late larval and post-larval forms of benthic clams, gastropods, polychaetes, and crustaceans associated with hydrothermal vents were collected in the low altitude tows extending 100–200 mab. This indicates that post-larval stages may play an active role in dispersal of hydrothermal vent species.

This series of long horizontal MOCNESS tows taken close to the sea floor at 2000 m depth in the Guaymas Basin demonstrates the feasibility of doing precision low altitude towing for plankton over this and other hydrothermal vent areas for detailed analyses of structure of benthopelagic fauna.

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Faunal Distributions

Patterns in the distributions of individuals and populations have been identified for species occurring at seafloor hydrothermal vents and for communities of vent species along ridge segments. From these patterns, we can begin to appreciate the spatial scales over which processes controlling species distributions are operative:

Within a vent community, a pattern of zonation that correlates with gradients of water temperature and chemistry is observed. This zonation is on the scale of centimeters to meters and reflects physiological tolerances and requirements compounded by biological interactions such as recruitment, competition, and predation.

Among vent sites on a ridge segment, vent communities share the same pool of species, but the relative abundance of each species varies from one site to another. This variation is primarily an expression of chance events during recruitment and colonization, maturity of the vent community, and the local hydrographic regime.

On a basin-wide scale, the fauna of vent communities represent biological continua, where gradual morphological and genetic differentiation in species is correlated with increasing distance between vent sites. Dispersal abilities of larvae and adults, regional hydrographic regimes, and spacing of hydrothermal circulation are important determinants of the degree of species and community differentiation.

Differentiation of faunal assemblages at vents occurs at a global scale. The North American landmass discontinuity of the ridge axis in

the Eastern Pacific isolates a Northeast Pacific faunal assemblage (the vent fauna of Explorer, Juan de Fuca, and Gorda Ridges) from an East Pacific Rise/Galapagos faunal assemblage. Western Pacific fauna of the Marianas and Lau Back Arc Basins comprise a third, distinctive assemblage and a fourth assemblage is found on the Mid-Atlantic Ridge. Geographic isolation of populations over a geological time frame is the most likely explanation for these divergent assemblages.

The spatial scales and biological differentiation outlined above serve as a template for basin-wide, multi-segment studies of benthic communities associated with hydrothermal activity. Essential components of future work should focus on refinement of our understanding of species' distributions and expansion of efforts to measure genetic relatedness among populations along and between ridge axes. Studies of age-specific growth, reproduction and mortality and of the abundance, distribution, and nutrition of dispersal stages of vent species will improve our understanding of population dynamics and the processes that control species distributions.

Trophic Studies

The trophic structures of vent communities are not uniform. At the most basic level, there is a dichotomy between vents dominated by symbiotic primary producer/primary consumer populations (tubeworms and mollusks) and vents dominated by free-living primary producer and grazing populations (shrimp).

Among symbiont-dominated communities, non-symbiont species are relatively independent of symbiont primary production. Instead, non-symbiont species -- polychaetes, limpets, small crustaceans, etc. --

depend primarily on free-living chemoautotrophic bacteria growing on surfaces or suspended in the water column. Surface-derived photosynthetic carbon is not a significant source of nutrition for vent species.

Patterns in the stable isotopic compositions of vent and seep symbiont species provide some insight into important sources of carbon and nitrogen within chemosynthetic communities and point to fundamental differences in carbon isotopic fractionation between bivalve mollusk and tubeworm symbioses.

Distinctive isotopic compositions of chemosynthetically-derived organic material have potential as tracers of the contribution of chemosynthetic production to the surrounding seafloor and water column non-vent communities. Isotopic compositions of vent invertebrates may also prove useful in understanding microhabitat heterogeneity in species' distributions within vent sites in response to the availability of various isotopic pools of carbon and nitrogen.

Vision and Light

Rimicaris exoculata, a "blind" shrimp that colonizes black smoker chimneys at Mid-Atlantic Ridge vents, has a novel photoreceptor adapted for detection of very dim light. Discovery and description of this 'eye' led to the hypothesis that the shrimp are detecting photons emitted by the plume of hot (350°C) vent water. Subsequent field programs using a sensitive electronic CCD camera demonstrated a measurable photon flux emitted from hot water at the base of hydrothermal plumes. Black body radiation could account for the levels of light detected by the camera. A more complete characterization of

spectral photon flux would confirm this hypothesis and identify other sources of light superimposed on the black body emission spectrum. Calculations based on estimated photon flux, absorption characteristics of long-wavelength absorbing photosynthetic pigments, photon flux requirements for photosynthesis, and absorption characteristics of seawater suggest that the potential for geothermally-driven photosynthesis at deep-sea hydrothermal vents cannot be dismissed.