



Particulate matter as a food source at a nascent hydrothermal vent on the Juan de Fuca Ridge

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Introduction

One of the most striking aspects of deep-sea hydrothermal vent communities is their reliance on local microbial chemolithoautotrophic production. This microbial primary production takes place both in free-living form and as endosymbiotic associations with metazoan hosts (Karl, 1995). While nutritional relationships between symbionts and their faunal hosts have received considerable attention, little is known about food sources consumed by the non-symbiont-containing (NSC) fauna. Yet, these organisms compose a major part of the vent faunal biomass (e.g.: Sarrazin & Juniper, 1999). They rely on organic matter consumption, either through suspension- and deposit-feeding or through predation. The biochemical composition and nutritional quality of sources of particulate organic matter available to vent suspension- and deposit-feeders remain poorly described. Potential sources include free-living bacteria (both autotrophic and heterotrophic), protozoa, meiofauna, as well as non-living detrital organic matter from the vent habitat and from the pelagic zone. The vent ecosystem is known to be highly dynamic in habitat characteristics and community composition (e.g.: Sarrazin & Juniper, 1999). The biochemical composition of the particulate food sources available to consumers is likely to vary accordingly.

A first goal of this contribution is to describe the composition and nutritional value of the particulate matter available to vent deposit- and suspension-feeders, using biochemical analyses and microscopic observations. A second goal is to assess the temporal changes in the biochemical composition and nutritional quality of organic matter at a developing hydrothermal vent. The initiation of new hydrothermally active sites by a volcanic eruption at

Axial Volcano (Juan de Fuca Ridge) in January-February 1998 (Embley et al., 1999), provided the opportunity to study this dynamic aspect of the vent food web. We present here results of the biochemical composition and nutritional value of particulate matter from one of these sites, Marker 33 Vent, from eight months to 17 months after its creation.

The C/N ratio and available protein content are frequently used descriptors of the nutritional quality of particulate matter for metazoan consumers (Mayer et al., 1986; Wotton, 1990; Grémare et al., 1997). Because bacteria form an important, nutritionally-rich food source for suspension- and deposit-feeders (Lopez & Levinton, 1987), and since bacteria are the primary producers at the base of the vent consumer food chain, we also enumerated particle-associated DAPI-staining bacterial cells as another indicator of the nutritional quality of particulate matter. In addition, we used stable nitrogen isotope analyses to evaluate the significance of the observed changes in the composition of the particulate matter for the food web.

Materials and methods

Marker 33 Vent is located on the January-February 1998 lava flow in the caldera of Axial Volcano, Juan de Fuca Ridge (45°56'N; 130°00'W; depth ~1500m). A first visit in September 1998 revealed a ~20m-diameter site on the basaltic seafloor covered with white microbial mat, on which polychaetous deposit feeders (alvinellids and polynoids) were observed feeding. No other vent macrofauna was observed in 1998. Other consumer species and vestimentiferan tube worms had colonized the site by the time of the next visit in June-July 1999, and were still present in June-July 2000.

Particulate matter (PM) and fauna were sampled with the remotely operated vehicle ROPOS in September 1998, June-July 1999, and June-July 2000 (fauna only). Two PM samples were collected in 1998 and 1999 from the same location in the central part of the faunal assemblage, using a suction-sampling device that pumped water through 2 l acrylic jars. Two layers of 200 µm Nitex nylon mesh at the outlet of the jar concentrated particles within the jar. The intake hose of the suction sampler was positioned within the faunal assemblage by the ROPOS 5-function manipulator. Following PM sampling, faunal specimens were collected using the suction sampler or the vehicle's 7-function manipulator. All samples were kept at ambient bottom temperature (~2°C) in closed jars or in the ROPOS biobox until they were brought to the surface, where they were frozen (-80°C). Aliquots of PM samples were preserved in cacodylate-buffered glutaraldehyde (3% final concentration) for scanning electron microscopic (SEM) observations and in seawater-buffered formalin (7% final concentration) for DAPI staining and epifluorescence microscopy.

Glutaraldehyde-preserved samples were observed with a Hitachi S-2300 SEM, which was also used for energy-dispersive X-ray analysis (EDX) of freeze-dried aliquots from frozen samples. Formalin-preserved samples were DAPI-stained, and DAPI-staining cells counted in replicate by epifluorescence microscopy. Frozen samples were thawed and collected on pre-combusted GF/F glass fibre filters. Organic carbon and nitrogen contents were measured on freeze-dried samples with a Carlo-Erba CN analyser. Available protein content was measured in triplicate as in Mayer et al. (1986), using freeze-dried samples. The stable nitrogen isotope composition of gut content-cleared, freeze-dried animals was analysed using a Micromass Isoprime isotope ratio mass spectrometer, and is reported as follows, using atmospheric N₂ as standard:

$$\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}} / {}^{15}\text{N}/{}^{14}\text{N}_{\text{standard}}) - 1] \times 1000 (\text{‰})$$

Results and Discussion

Scanning electron and epifluorescence microscopic observations revealed that the PM from Marker 33 Vent was a complex mixture of abundant mineral grains, coccoid and filamentous bacterial forms, remains of diatom frustules, and amorphous debris. This is similar to PM from other post-eruptive nascent vent sites at Axial Volcano (unpublished observations).

The biochemical composition (organic carbon and nitrogen content, C/N ratio, available protein content and DAPI-staining cell counts) of the particulate matter from Marker 33 vent in 1998 and 1999 is summarized in Table 1. The elemental composition of the PM, analysed with EDX on a single sample in 1998, was largely dominated by sulphur (66-71 weight %). In all samples (1998 and 1999), organic carbon contents (0.5 to 9.9%) and nitrogen contents (0.1 to 1.2%) were usually low, but highly variable. The C/N

ratio varied between 5.3 and 9.8, indicative of a relatively labile, protein-rich material. This C/N ratio is similar to that of marine bacteria (~4-6; Parsons, 1975), and lower than that reported for suspended particulates at East Pacific Rise hydrothermal vents (10-15; Comita et al., 1984) and from nearshore and deep-sea sediments (10-25; Parsons, 1975; Comita et al., 1984; Grémare et al., 1997). Available protein content (2.9 - 30.9 mg g⁻¹ dry weight) was of the same order as that reported for shallow-water coastal sediments (e.g.: Mayer et al., 1986; Grémare et al., 1997). DAPI-staining cell counts (2.5X10⁹ - 1.9X10¹⁰ cells g⁻¹ dry weight) were at the upper range of values reported for the surface of hydrothermal sulphide chimneys (7.8X10⁸ - 1.1X10⁹ cells g⁻¹ dry weight; Chevaldonné, 1996), but further comparison is hindered by the fact that Marker 33 Vent and sulphide chimneys are different types of habitats.

Table 1. Biochemical composition (organic carbon and nitrogen content, C/N ratio, available protein content and DAPI-staining cell counts) of the particulate matter from Marker 33 Vent in 1998 and 1999. Values are mean (range). T-test p-values are for inter-annual comparisons (n=2, except for DAPI-staining cell counts in 1998 where n=1). DW = dry weight.

	1998	1999	T-test p-value
% organic carbon	1.6 (2.6)	4.7 (6.6)	0.0273
% nitrogen	0.3 (0.4)	0.6 (0.8)	0.0524
C/N ratio	6.3 (1.9)	8.6 (2.6)	0.0021
Proteins (mg g ⁻¹ DW)	6.4 (6.4)	16.8 (28.1)	0.1897
Proteins (mg g ⁻¹ C)	627 (1051)	203 (287)	0.3448
DAPI-staining cells x10 ⁹ g ⁻¹ DW	18.6	3.6 (2.3)	
DAPI-staining cells x10 ⁹ g ⁻¹ C	1205	114 (98)	

The large range in the biochemical composition of samples from a same year indicates that the PM at Marker 33 Vent was highly variable in nutritional quality. This has important implications for the trophic ecology of vent consumer species. Further evidence for a heterogeneous particulate food supply is provided by a stable isotope-based study of the trophic ecology of suspension- and deposit-feeding alvinellid polychaetes from Juan de Fuca Ridge hydrothermal vents (Levesque et al., submitted). This latter study found that intra- and interspecific trophic resource partitioning is common, indicating that species and individuals of a same species utilize different sources of dietary organic matter. Individuals exploiting more nutritionally-rich sources of organic matter will have a trophic advantage over other organisms.

The biochemical composition of the PM changed drastically between 1998 and 1999. The organic content of the PM increased, with the organic carbon content tripling

(1.6% to 4.7% dry weight; $p=0.0273$), and the nitrogen content doubling (0.3% to 0.6% dry weight; $p=0.0524$). This is reflected in the available protein content (protein g^{-1} dry weight), which tended to increase during the same period (from 6.4 to 16.8 mg g^{-1} dry weight; non-significant change). The mean C/N ratio increased significantly, from 6.3 to 8.6 ($p=0.0021$), as reflected in the available protein content g^{-1} C, which tended to decrease (from 627 to 203 mg g^{-1} C; non-significant change). DAPI-staining cell counts (both cells g^{-1} dry weight and cells g^{-1} C) drastically decreased between 1998 and 1999. However, this observation remains uncertain, because only one sample was processed for cell counts in 1998. Assuming that bacteria contain 10^{-14} g carbon cell^{-1} (Williams & Carlucci, 1976) and have a C/N ratio of 4, bacteria contributed 1.2% of the PM organic carbon and 1.7% of the nitrogen in 1998, compared to only 0.1% of the organic carbon and 0.3% of the nitrogen in 1999. This provides a quantitative estimate of the decreased bacterial contribution to the organic matter pool between 1998 and 1999 at Marker 33 Vent. In summary, while the organic content of the particulate matter at Marker 33 Vent increased between 1998 and 1999, the nutritional quality of this organic matter decreased during the same period, based on the C/N ratio, DAPI-staining cell counts and, to a lesser extent, available protein content.

The accumulation of lower quality organic matter at Marker 33 Vent could reflect a gradually increasing contribution of autochthonous and allochthonous detritus to the local PM pool. In 1998, the particulate matter present at Marker 33 Vent had to be recently produced or accumulated, since the site was completely covered by a lava flow eight months before. This new PM was therefore likely dominated by local free-living microbial primary production, with a contribution from pelagic material that had sedimented during the eight-month period. In June-July 1999, in addition to local free-living bacterial production, the PM at Marker 33 Vent was also composed of detrital organic matter that had accumulated over the previous 17 months, derived from local sources (debris and mucus) and sedimented from the pelagic zone. Therefore, detritus from both local and pelagic sources likely made a greater contribution to Marker 33 vent PM in 1999 than it did in 1998, providing an explanation for the observed changes in C/N ratio, available protein content and DAPI-staining cell counts. Pelagic sediments collected at three different locations on Axial Volcano are characterized by a high C/N ratio (mean=19), low available protein content (mean=31.7 mg g^{-1} C) and low DAPI-staining cell counts (mean= 65×10^9 cells g^{-1} C; unpublished data), which indicates that a higher proportion of sediments in the PM pool at Marker 33 in 1999 could indeed explain the observed changes. Accumulation of detritus is a process commonly occurring during succession of biological communities (Keeton & Gould, 1993), but it has never been verified at vents.

These observations provide the basis for a hypothesis whereby, as a vent site ages, a higher proportion of the suspension- and deposit-feeder's diet would come from heterotrophic pathways (detritus), whereas direct consumption of autotrophic microbial biomass would become less important by comparison.

This hypothesis is supported by the stable nitrogen isotopic composition of the consumer fauna at Marker 33 Vent. The stable nitrogen isotope composition ($\delta^{15}\text{N}$) of an animal can be used as an indicator of its trophic position, since nitrogen is fractionated in trophic transfers such that the $\delta^{15}\text{N}$ of a consumer is $\sim 3\text{--}4\text{‰}$ higher than that of its food sources (Minawaga & Wada, 1984). At Marker 33 Vent, the $\delta^{15}\text{N}$ of consumer fauna increased during the 1998-2000 period, mostly between 1999 and 2000. During the two-year period (1998-2000), out of the seven consumer species analysed for their $\delta^{15}\text{N}$, 5 deposit- and suspension-feeding species (the polychaetes *Amphysamytha* cf. *galapagensis* Zottoli, 1983, *Paralvinella palmiformis* Desbruyères & Laubier, 1986 and *Paralvinella pandorae* Desbruyères & Laubier, 1986, and the gastropods *Depressigyra globulus* Warén & Bouchet, 1989 and *Lepetodrilus fucensis* Mc Lean, 1988) significantly increased in $\delta^{15}\text{N}$ by 0.6 to 2.1‰ ($p<0.05$), while the other two species (the polynoids *Branchinotogluma* sp. and *Lepidonotopodium piscesae* Pettibone, 1988) did not change significantly in $\delta^{15}\text{N}$ (Table 2). Because the stable isotopic composition of an animal integrates that of its past diet, this increase in $\delta^{15}\text{N}$ reflects the gradual change in $\delta^{15}\text{N}$ following consumption of ^{15}N -enriched organic matter.

Because of the 3-4‰ trophic shift, detritus from animals have a higher $\delta^{15}\text{N}$ than primary producers. Sedimentary organic matter is also well known to be enriched in ^{15}N compared to vent biota (e.g.: Conway et al., 1994). Thus, a higher proportion of detrital organic matter from either local or sedimentary sources in the diet of Marker 33 Vent animals would both result in a higher $\delta^{15}\text{N}$. The increase in $\delta^{15}\text{N}$ of the consumer fauna at Marker 33 Vent therefore supports the hypothesis of a heterotrophication of the diet of deposit- and suspension-feeding species, but it does not indicate whether this ^{15}N -enriched organic matter is of autochthonous and/or allochthonous origin.

To correctly evaluate the trophic position of an organism using stable nitrogen isotopes, the $\delta^{15}\text{N}$ of primary producers at the base of the food chain must be known. It is theoretically possible that the increase in $\delta^{15}\text{N}$ of consumers observed between 1998 and 2000 could be the result of a different fractionation of assimilated N by free-living primary producers, and not of an increasing proportion of ^{15}N -rich heterotrophic organic matter in their diet. For obvious sampling limitations, we could not measure the $\delta^{15}\text{N}$ of free-living microbial primary producers. However, the stable nitrogen isotope composition of the endosymbiont-containing vestimentiferan tube worm *Ridgeia piscesae* Jones, 1985 could be an indicator of the $\delta^{15}\text{N}$ of free-living

Table 2. Stable nitrogen isotopic composition ($\delta^{15}\text{N}$) of consumer species from Marker 33 Vent, 1998-2000. Values are means (SD) from 5 individuals of each species. An asterisk (*) indicates a significant change (ANOVA, $p < 0.05$) in $\delta^{15}\text{N}$ from the previous year.

Species	1998	1999	2000
<i>Amphysamytha</i> cf. <i>galapagensis</i>		6.5 (1.3)	7.1 (0.6)*
<i>Branchinotogluma</i> sp.	7.7 (0.9)	7.7 (1.5)	6.9 (2.4)
<i>Depressigra globulus</i>		7.5 (0.8)	9.6 (0.0)*
<i>Lepetodrilus fucensis</i>		5.5 (0.0)	7.2 (1.0)*
<i>Lepidonotopodium piscisae</i>	7.8 (1.9)	7.1 (2.7)	7.6 (2.6)
<i>Paralvinella palmiformis</i>	6.3 (0.6)	5.6 (0.6)	7.1 (0.7)*
<i>Paralvinella pandorae</i>	6.2 (0.2)	5.6 (1.2)	7.3 (1.0)*

primary producers. This is supported by the correlation between the $\delta^{15}\text{N}$ of *R. piscisae* and the $\delta^{15}\text{N}$ of many consumer species observed across several Juan de Fuca Ridge vent sites (C. Levesque, unpublished data). In the present study, the $\delta^{15}\text{N}$ of *R. piscisae* actually decreased by 1.6‰ during the period considered (data not shown). This suggests that the increase in $\delta^{15}\text{N}$ of Marker 33 Vent consumers does not result from an increase in the $\delta^{15}\text{N}$ of free-living primary producers, but rather from a higher proportion of ^{15}N -rich detrital organic matter in their diet.

Conclusion

This study revealed several interesting aspects of particulate matter food sources at Marker 33 Vent. The observed spatial and inter-annual variability in the biochemical composition and nutritional quality of PM within this site has important implications for consumer species, both in terms of dietary flexibility and resource partitioning. The observed decrease in PM nutritional quality and the generalized shift in $\delta^{15}\text{N}$ of consumer species provide evidence for an increasing contribution of detritus in the diet of consumers, leading to heterotrophication of the food web as the vent site ages. Because of the limited scope of this study (a single study site, small number of samples), these results should be regarded as preliminary. Further studies are currently being conducted to understand the composition, trophic role and temporal dynamics of PM at vents and to test, on a broader scale, the hypothesis of the heterotrophication of organic matter during early succession at deep-sea hydrothermal vents.

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