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Nematode communities associated with tubeworm and mussel aggregations on the East Pacific Rise

Julia ZEKELY[1], Sabine GOLLNER[1], Cindy Lee Van DOVER[2], Breea GOVENAR[3], Nadine Le BRIS[4],
Hans Leo NEMESCHKAL[5] and Monika BRIGHT[1]
(1) *Department of Marine Biology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria.*
Tel. +43-14277-57210, E-mail: j.zekely@gmx.at
(2) *Biology Department, College of William & Mary, Williamsburg, Virginia 23187, USA*
(3) *Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA*
(4) *Département Etude des Ecosystèmes Profonds, Ifremer, Centre de Brest, BP 70, 29280 Plouzané, France*
(5) *Department of Theoretical Biology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria*

Abstract: The meiobenthic nematode community of three different vent sites at the East Pacific Rise was studied in order to determine whether the abundance, species richness, diversity and trophic structure were similar. The sites Tica and Riftia Field were dominated by the tubeworm *Riftia pachyptila,* and the Buckfield site was dominated by the mussel *Bathymodiolus thermophilus*. The nematode communities of all three sites were low in abundance (< 1 up to 46 ind. 10 cm^{-2}), except one sample from Tica with almost 1000 ind. 10 cm^{-2}. The communities at all sites consisted entirely of primary consumers, mostly deposit feeders. Species richness and Shannon-Wiener diversity indices were low and similar at both tubeworm sites and slightly but significantly higher at the mussel site. Multivariate analysis revealed that the species dissimilarity among the three sites was greater than 50 %, indicating distinct communities at each site.

Keywords: Hydrothermal vent • Meiobenthos • Nematodes • *Riftia pachyptila* • *Bathymodiolus thermophilus* • East Pacific Rise

Introduction

Ecological community studies address the distribution and abundance of organisms. The spatial and temporal heterogeneity of a habitat can play an important role in the structure of a community (Begon et al., 1999). Most ecological studies at hydrothermal vents have focused on mega- and macrofauna. The few studies of the meiofauna, (animals and protists passing through a 1 mm and retained on a 63 µm mesh-sized sieve) (see Giere, 1993), have indicated that the meiobenthos is a significant part of the hydrothermal vent community (Dinet et al., 1988; Shirayama, 1992; Vanreusel et al., 1997; Zekely et al., 2006). Nematoda is one of the dominant meiobenthic taxa and is known from a variety of terrestrial, freshwater, and marine shallow-water and deep-sea habitats (Giere, 1993). Vanreusel et al. (1998) were the first to describe the structure of a vent nematode community from mussel aggregations at soft-sedimented hydrothermal vents at the North Fiji Back-Arc Basin. The abundance and composition of nematodes were significantly different between active sites, inhabited by *Bathymodiolus brevior* Cosel & Métivier, 1994 and inactive vent sites, devoid of megafauna (Vanreusel et al., 1997).

The aim of this study was to investigate and compare the nematode communities of three hydrothermal vent sites on the northern East Pacific Rise, which differ in environmental characteristics, including the occurrence of the structuring large animals *Riftia pachyptila* Jones, 1981 and/or *Bathymodiolus thermophilus* Kenk & Wilson, 1985. These megafaunae create a unique three-dimensional habitat with considerable interstitial space in which small macro- and meiobenthic animals, including nematodes, live. They are a suitable structural habitat for biodiversity and community structure comparisons at hydrothermal vents.

Material and Methods

Nine megafaunal aggregations were sampled, three each at the sites Riftia Field (9°50.705´N, 104°17.493´W, 2500 m depth) and Tica (9°50.447´N, 104°17,493´W, 2500 m depth) (both dominated by the tubeworm *Riftia pachyptila*, but *Bathymodiolus thermophilus* mussels also occurred at Tica) and three at the site Buckfield (11°24.90´N, 103°47.20´W, 2480 m depth), where the mussel *B. thermophilus* was the dominant megafaunal species (Table 1). All megafaunal aggregations assembled on bare basalt and small amount of sediment, mostly particulate organic matter with few mineral grains, accumulated between tubes and/or shells. In 2002, chemical concentrations of $\sum H_2S$ and ferrous iron, pH and temperature were measured at the collection sites *in situ* with the *"Alchimist"* (Le Bris et al., 2005). At Riftia Field the maximum temperature was 23°C, sulfide concentrations were up to 35 µM $\sum H_2S$, minimum pH was 5, and dissolved ferrous iron concentrations were as high as 42 µM. At Tica, maximal temperatures were similar to Riftia Field (maximum 18°C), but the chemistry of the fluid was significantly different (maximal sulfide concentrations up to 176 µM $\sum H_2S$, neutral pH, ferrous iron was not detected) (Le Bris et al., 2005; Table 1). At the time of collection of mussels at Buckfield, maximum temperatures were approximately between 4 and 10°C (Van Dover, pers. obs.). No chemical measurements were conducted during sampling at Buckfield.

Quantitative tubeworm samples at Riftia Field and Tica were taken using DSV *Alvin* and a hydraulically actuated collection net named the "Bushmaster Jr." in December 2001 and December 2002 (sampling area up to 60 cm in diameter; for details see Govenar et al., 2005). The mussel samples at Buckfield were collected with the "mussel pot" sampling device (531 cm^2 collection area) with DSV *Alvin* in December 2001 (for details see Van Dover, 2002). On board the ship, megafaunal aggregations were immediately disassembled and rinsed 3 times with 10 µm filtered seawater to wash off associated fauna and sediment. To extract the meiofaunal community, the samples were washed through a series of sieves (63 µm, 250 µm, 1 mm sieve size), fixed in 4% buffered formalin for 24 h, and stored in 70% ethanol. The nematode community was sorted and individuals were counted under a dissection microscope. If present, 300 nematodes per sample were haphazardly chosen and mounted in glycerin for identification to the lowest possible taxon level.

Due to the different sizes of the sampled areas (300 cm^2 to 1300 cm^2 for tubeworm aggregations and 531 cm^2 for mussel aggregations) and in order to compare these large sample areas with each other and to other meiofauna studies, we standardized the abundance to 10 cm^2 surface area. Individual biomass (µg wet weight) of nematode species was estimated according to Andrassy (1956) [wt (µg) = length (µm) x width^2 (µm) / 1600000; wt = µg wet weight, L = length (from anterior to posterior end) and W = maximum diameter of body]. All identified specimens were measured and the total biomass of the nematode community was estimated by the summation of the mean biomass of

Table 1. Characteristics of collection sites (*measured in 2002; Le Bris et al., 2005) and nematode communities according to sites.
Tableau 1. Caractéristiques des sites de prélèvement (* mesuré en 2002; Le Bris et al., 2005) et des communautés de nématodes de chaque site.

	Riftia Field	Tica	Buckfield
Maximum temperature [°C]	23*	18*	10
Maximum $\sum H_2S$ [µM]	35*	176*	N/A
Maximum ferrous iron [µM]	42*	0*	N/A
Minimum pH	4.4*	5.7*	N/A
Dominant megafauna	*R. pachyptila*	*R. pachyptila*	*B. thermophilus*
Total abundance	11 - 573	951 - 28369	51 - 66
Abundance 10 cm^{-2}	< 1 - 7	< 16 - 946	1 - 2
Species richness	1 - 3	3 - 4	8 - 9
Shannon-Wiener diversity	0.4 - 0.7	0.3 – 0.5	1.5 – 1.7
Pielou's evenness	0.3 - 0.9	0.2 - 0.4	0.7 - 0.8
Biomass [mg wet weight 10 cm^{-2}]	< 0.001 – 0.001	0.002 – 0.16	< 0.0001

each measured species by the total abundance of each species in each sample.

The trophic status of the nematodes was determined by morphology and classified into primary consumers, parasitic and predatory secondary and tertiary consumers, following Wieser (1953).

To illustrate the degree of heterogeneity and dominance patterns, k-dominance curves were constructed for each sample by plotting the relative abundance of each species against the decreasing rank of dominant species. To describe the nematode community structure, species richness (S), Pielou's evenness index (J´), and Shannon-Wiener diversity index ($H'_{\log e}$) were calculated. The Students t-test was used to test significant differences in abundance (square-root transformed) and species richness (square-root transformed). Due to relative small number of samples, bootstrapping (1000 resamplings each, two-sided test; Nemeschkal, 1999) was used to test for significant differences in abundance, species richness, and Shannon-Wiener diversity indices among the three sites (Nemeschkal, 1999). Results from statistical analysis were Bonferroni-corrected ($p = a/n$; $a = 0.05$). Hierarchical clustering was used to compare communities of the three sites. The similarity matrix for cluster analysis was generated using Bray-Curtis similarity values calculated from square-root transformed, standardized abundance data. All univariate indices and multivariate measures were performed using the PRIMER v5 package (Plymouth Marine Laboratory; Clarke & Gorley, 2001).

Results and Discussion

At the East Pacific Rise striking spatial patterns of typical megafauna assemblages along a gradient of hydrothermal fluid flux are common (e.g. Shank et al., 1998). Hydrothermal vent sites of vigorous to moderate diffuse flow such as Tica, Riftia Field, and Buckfield are often densely populated by large aggregations of tubeworms and/or mussels. Assembled on bare basalt, the tubes or shells and byssal threads of these animals provide a highly structured three-dimensional habitat in which little sediment accumulates. Nematodes, one of the most divers and abundant meiofaunal taxa in virtually all marine benthic habitats, were found to be a consistent but small part of the associated epifaunal community. In this study, the diversity of nematodes was extremely low with very few species present in low abundance and biomass. The presence of these impoverished nematode communities at the northern East Pacific Rise stands in contrast to those of sedimented vent sites from other biogeographical regions, such as from the North Fiji Back-Arc Basin (Vanreusel et al., 1997). Multivariate analyses of community structure revealed that each site, differing in environmental characteristics, harboured its own distinct community. Furthermore, the similarity of both tubeworm sites was higher with univariate measures than the similarity of each tubeworm site to the mussel site.

Inferred from mouth structure analyses, the nematode communities associated with tubeworms and mussels were entirely comprised of deposit feeders; whereas in other habitats, nematode communities are dominated by primary consumers but consumers of higher trophic levels are usually present (see Giere, 1993). No predators were collected this study.

In eight out of nine samples, abundances ranged from only < 1 to 46 ind. 10 cm^{-2}, with biomasses ranging from 0.001 to 0.006 mg 10 cm^{-2} (Table 1). Although statistically not significantly different, one exceptional sample from

Table 2. Relative abundance (%) of nematode species occurring at Tica (TC), Riftia Field (RF) and Buckfield (BF).
Tableau 2. Abondance relative (%) des espèces de nématodes présents à Tica (TC), Riftia Field (RF) et Buckfield (BF).

	TC1	TC2	TC3	RF1	RF2	RF3	BF1	BF2	BF3
Species from three sites									
Thalassomonhystera fisheri	88	94	84	60		10	48	45	49
Species from two sites									
Chromadorita sp. 1			< 1				7	5	1
Monhysteridae sp. 1	7	3	15				21	13	8
Monhysteridae sp. 2	5	3	< 1	40	100	90			
Species from one site									
Anticoma sp. 1							2	3	
Daptonema sp. 1						< 1			
Leptolaimus sp. 1							6	3	7
Megadesmolaimus sp. 1							6	5	6
Paracanthonchus sp. 1							3	5	6
Paralinhomoeus sp. 1								7	1
Theristus sp. 1							7	14	22

Tica (946 ind. 10 cm^{-2}; 0.164 mg 10 cm^{-2}; Table 1) fell within the well-known range of nematode abundance from other shallow-water or deep-sea habitats and might point to a patchy distribution (see Giere 1993) at hydrothermal vents. The only species co-occurring at all sites was *Thalassomonhystera* sp. 1. It dominated relatively consistently between 84 and 94% to the total community at Tica and between 45 to 49% at Buckfield, while its contribution between 0 and 60% at Riftia Field was highly variable (Table 2).

As expected, univariate and multivariate measures of community structure point to distinctly different communities along an environmental gradient. Although inferred from a total of nine samples only and a few physico-chemical data available for these sites, it appears that the nematode communities were affected by abiotic key factors such as temperature and sulfide concentrations, known to be critical for hydrothermal vent animals. Nevertheless, the dominant megafauna structuring the habitat might have a basic influence on its associated community, since the degree of similarity was higher between both tubeworm sites than between those and the mussel site. The tubeworm communities, at the time of sampling, were exposed to similar maximal temperatures near 20°C, in contrast to the mussel bed at Buckfield with maximum temperatures ranging from 10°C to slightly above ambient (~ 2°C) temperatures (Table 1). Also, the age of the communities, quite different between the three sample sites, might have an influence on the structure of the nematode communities. The mussel bed Buckfield is older (> 10 yrs; Dreyer et al., 2005) and exhibited a more divers macrobenthic community than Riftia Field (< 10 yrs; Van Dover, 2000), while Tica is the youngest habitat (< 7 years; Van Dover, 2000) with the lowest diversity. Univariate measures of community structure revealed clear differences between the tubeworm and mussel bed communities. A total of only three species from Riftia Field and four species from Tica were identified. The few species found, were unevenly distributed among samples and sites. Species richness and Shannon-Wiener diversity were low and not significantly different between Riftia Field and Tica (Table 3). In contrast, nine species were found at Buckfield, and species richness and Shannon-Wiener diversity were significantly higher than both of the tubeworm sites (Table 3). The same trends held true for Pielou’s evenness. The more pronounced similarity among the both tubeworm-dominated nematode communities as well as the distinct community associated with the mussel bed was also evident in the k-dominance curves (Fig. 1).

Multivariate analyses (SIMPER) revealed that the species dissimilarity between sites was > 50% between Tica and Buckfield, > 60% among Tica and Riftia Field and > 80% between Riftia Field and Buckfield (see also Fig. 2).

Table 3. Bootstrapping and Students t-test (two-sided, t_{4df} = 2.776, in parentheses) was used to test for differences in abundance (Ab, ind. 10 cm^{-2}) and species richness (S) between the three sites (TC = Tica; RF = Riftia Field; BF = Buckfield). Bootstrapping was used to test for differences in Shannon-Wiener diversity (H´$_{\log e}$). Results are given prior Bonferroni corrections. * Significantly different (after Bonferroni corrections; $p < 0.05$).

Tableau 3. Bootstrap et test t de Student (bilatéral, t_{4ddl} = 2.775, entre parenthèses) utilisés pour tester les différences d’abondance (Ab, ind. 10 cm^{-2}) et de richesse spécifique (S) entre les trois sites (TC = Tica; RF = Riftia Field; BF = Buckfield). Bootstrap utilisé pour tester les différences d’indices de diversité de Shannon-Wiener (H´$_{\log e}$). Les résultats sont donnés avant la correction de Bonferroni. * Différence significative (après la correction de Bonferroni ; $p < 0,05$).

	TC vs RF	TC vs BF	RF vs BF
Ab	0.026 (1.487)	0.026 (1.491)	0.744 (0.015)
S	0.058 (1.91)	0.003* (-10.035)	0.027* (6.849)
H’$_{\log e}$	0.826	0.003 *	0.003 *

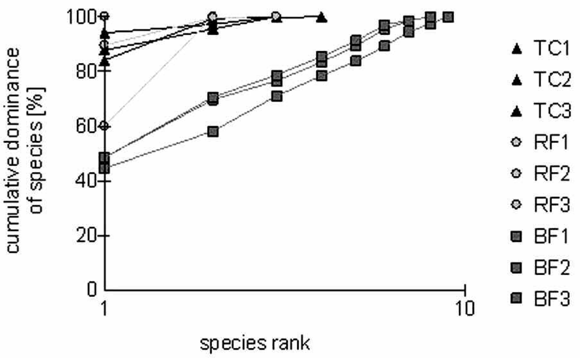

Figure 1. Mean cumulative dominance of nematode species from the three sites (TC = Tica; RF = Riftia Field; BF = Buckfield); relative abundances of species were plotted against species rank (i.e. number of species) for each sample.

Figure 1. Dominance cumulée moyenne des espèces de nematodes des trois sites (TC = Tica; RF = Riftia Field; BF = Buckfield) ; les abondances relatives des espèces sont représentées en fonction du rang des espèces pour chaque prélèvement.

These patterns were also supported by an analysis of similarity (ANOSIM; global R = 0.942, p = 0.4). The similarity between Tica tubeworm and the Buckfield mussel communities was due to the homogeneity among the samples with Bray-Curtis similarity values > 85% at Tica and > 80% at Buckfield (Fig. 2). The latter mussel bed community was characterized by six out of nine species that lacked at the other sites. In contrast, the Riftia Field community was rather heterogeneous. The similarity among the Riftia Field samples was < 70%, due to the variable relative abundance

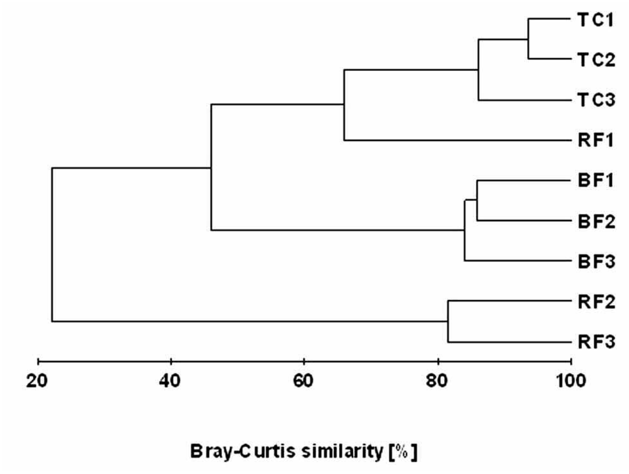

Figure 2. Hierarchical cluster diagram for group average linking based on Bray-Curtis similarities of nematode species from the nine samples (Tica samples: TC1, TC2, TC3; Riftia Field samples: RF1, RF2, RF3; Buckfield samples: BF1, BF2, BF3).

Figure 2. Dendrogramme de classification hiérarchique par groupement moyen fondé sur l'indice de similarité de Bray-Curtis des espèces de nématodes des neuf prélèvements (échantillons de Tica : TC1, TC2, TC3; échantillons de Riftia Field : RF1, RF2, RF3; échantillons de Buckfield : BF1, BF2, BF3).

of *Thalassomonhystera* sp. 1 (60%, 10% and 0%) and that one sample was composed entirely of a representative of undescribed genus of Monhysteridae sp. 2 (Table 2). The picture emerging from the available data on hydrothermal vent nematode distribution and community structure available so far points to a small community composed of a few, mostly yet undescribed species belonging to generalistic genera well known from many shallow-water and deep-sea environments. Deep-sea hydrothermal vent communities appear to have no strong affinities to other communities from sulfidic environments such as the 'thiobios' of sulfidic sediments, shallow-water vents, or cold seeps, despite the presence of reducing chemicals and hypoxia (Vanreusel et al., 1997).

In general, nematode diversity (species richness, Shannon-Wiener diversity) as well as nematode abundance is low at deep-sea hydrothermal vents. At a finer scale however, the East Pacific Rise communities from 9° and 11°N in this study were even less abundant than those from 21°N (Dinet et al., 1988), the Guaymas Basin (Dinet et al., 1988), and the Iheya Ridge (Shirayama, 1992), and less abundant and less diverse than those the North Fiji Back-Arc Basin (Vanreusel et al., 1997). The difference between the sites in this study and other hydrothermal vent sites maybe due to the substrate, but many other factors (e.g. the physico-chemical factors, the geographic locations) may contribute as well. Sediments through which hydrothermal fluid percolates or mussel aggregations accumulated above the sediments appear far more suitable for nematodes than mussel or tubeworm aggregations developing on bare basalt. While *in situ* experiments yet have to determine the exact location of nematode distribution within such aggregations either epibenthically on the tubes or shells and/or endobenthically within the little sediment accumulating between large animals, nematode communities are generally more diverse and more abundant in sediments than on hard substrate (Giere, 1993).

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