Obligate hydrothermal vent fauna at East Diamante submarine volcano (Mariana Arc) exploit photosynthetic and chemosynthetic carbon sources

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ABSTRACT: Two volcanic cones in the degraded caldera of East Diamante submarine volcano (Mariana Arc) were surveyed and sampled in April 2004 as part of the 'Submarine Ring of Fire 2004' expedition. The first images revealed hydrothermal venting within the photic zone and a striking overlap between chemosynthetic and photosynthetic communities. We used fatty acid biomarkers and bulk stable isotopes to determine the relative importance of chemosynthetic and photosynthetic material in the diets of invertebrates at 6 vent sites (Eastern Cone: Barnacle Beach and Black Forest; Central Cone: Floc Storm, Boulder Vent, Mid-cone, and Fe-Mn Crust) and 1 non-vent site (Central Cone: Aquarium). Principal components analysis revealed depth-related dietary differences: vent-obligate invertebrates at Barnacle Beach (~460 m) belonged to a purely chemosynthetic food web, non-vent organisms at Aquarium (179 m) had diets based on the products of photosynthesis, and most of the invertebrates at Black Forest (349 m) and Central Cone vent sites (247–288 m) had mixed chemosynthetic/photosynthetic diets. Carbon stable isotope ratios corroborated the photosynthetic input in the diets of the vent-obligate invertebrates. Long-chain polyunsaturated fatty acids were found in overlying waters and in detritus. We suggest that photosynthetic material arrives at East Diamante in the form of marine snow. Photic zone hydrothermal venting has now been found on several arc volcanoes in the western Pacific Ocean. Our study underscores the potential influence of benthic–pelagic coupling on trophic interactions and community structure for vent-obligate species in such settings.

KEY WORDS: Hydrothermal vent · Food webs · Trophic ecology · Fatty acids · Stable isotopes · East Diamante · Mariana Arc

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INTRODUCTION

Recent exploration of submarine volcanoes associated with intra-oceanic island arcs has permitted the study of hydrothermal vent communities in unusual environmental settings. These include chronic habitat perturbation by seafloor volcanism (Embley et al. 2006, Limén et al. 2006), exposure to acidity resulting from liquid CO_2 discharge (Tunnicliffe et al. 2009), the unique biogeographic situation of isolated arc

volcanoes (Van Dover et al. 2002), and highly contrasting hydrothermal conditions both on and between individual volcanoes (Davis & Moyer 2008, Huber et al. 2010). Of particular ecological interest is the occurrence of hydrothermal venting within the photic zone on arc volcanoes. Shallow oceanic venting (<200 m; defined by Tarasov et al. 2005) has thus far been observed on the Tonga Arc (Volcano 1; Comeault et al. 2010), the Kermadec Arc (Giggenbach volcano; Von Cosel & Marshall 2010), and at 3 locations on the Mariana Arc: Esmeralda Bank (Türkay & Sakai 1995), and the submarine volcanoes Maug (Embley et al. 2004) and East Diamante (present study). Shallow oceanic vents can be substantially populated by vent-obligate species known from deep-sea vents (>200 m; Comeault et al. 2010) and thus reveal the potential for a new type of vent ecosystem, one with links to both the photic zone and to benthic chemosynthetic production. While mixed chemosynthetic/photosynthetic diets have been documented at shallow hydrothermal vents in coastal settings (e.g. Kharlamenko et al. 1995), vent-obligate taxa are usually absent (reviewed by Tarasov et al. 2005). To date, only 1 study has examined the extent to which vent-obligate invertebrates utilize photosynthetic food sources on submarine volcanoes associated with intra-oceanic island arcs (Comeault et al. 2010).

Using fatty acid and stable isotope biomarkers, in combination with gut content analysis, Comeault et al. (2010) showed that vent-obligate invertebrates on Volcano 1 ingested both material produced at vents (chemosynthetic production) and material derived from photosynthetic organisms. There was some evidence that vent animals in the photic zone contained higher proportions of photosynthetically derived fatty acids than those found at greater depths. However, the samples from Volcano 1 were taken over a relatively small, homogenous sampling area with a limited depth range (100–200 m) and they were not taxonomically diverse. In our study, observations and sampling on East Diamante during the 'Submarine Ring of Fire 2004' expedition extended from vents well below the photic zone (~460 m) to the upper slopes of a volcanic cone where microbial mats overgrew coralline algae and sessile non-vent invertebrates (~180 m). A strong gradient in hydrothermal conditions and associated communities was observed over this depth range. Invertebrates from 7 taxa were collected, the majority of which were heterotrophic vent obligates, allowing us to evaluate the effects of depth, venting, and proximity to the photic zone on the diets of local invertebrates.

Food-web relationships and possible sources of organic carbon can be unclear at hydrothermal vents, and are often unknown before sampling takes place, particularly when sampling new sites and new species assemblages. In such situations, employing a suite of complementary methods can aid the determination of trophic linkages. The combination of fatty acids and stable isotopes to study hydrothermal vent ecology is particularly informative and has been used to investigate symbioses and heterotrophy in vent-obligate invertebrates at several sites worldwide (Pond et al. 1997a, 2000, 2002, Limén et al. 2008, Stevens et al. 2008, Comeault et al. 2010). Fatty acid analysis provides detailed dietary information, while stable isotope ratios of carbon and nitrogen can yield source material and trophic level estimates, respectively. Different groups of primary producers have characteristic fatty acid signatures that can be used to assess their utilization by consumers (Kelly & Scheibling 2012), and these biomarkers have helped distinguish between photosynthetic and chemosynthetic material in mixed diets (Comeault et al. 2010). Stable isotope ratios change in relatively predictable ways as material moves through food webs, with consumers being enriched in ^{15}N and ^{13}C by approximately 3.4 and 0.4‰, respectively, compared to their prey (Post 2002). In this study, we used fatty acid biomarkers and bulk stable isotopes to evaluate the importance of chemosynthetic versus photosynthetic material in the diets of invertebrates on East Diamante. We predicted that the relative importance of photosynthetic carbon would be positively related to the proximity of the photic zone and that organisms at the vent sites would have different diets from those at non-vent sites. To date, this is the most comprehensive study of mixed chemosynthetic/photosynthetic diets in vent-obligate invertebrates at shallow oceanic vents.

MATERIALS AND METHODS

Field sampling

The Mariana Arc submarine volcano East Diamante (15° 56′ N, 145° 40′ E) was explored and sampled from the RV 'Thomas G. Thompson' during the 'Submarine Ring of Fire 2004' expedition, using CTD surveys (tow-yo's) to identify areas of active hydrothermal venting, and 2 ca. 17 h dives on 3 to 4 April 2004 (R787, R788) using the

remotely operated vehicle 'ROPOS.' East Diamante has an old, partly degraded caldera with several large volcanic cones in the centre where hydrothermal venting is concentrated. The deepest sites sampled—Barnacle Beach (457–461 m) and Black Forest (348–349 m)—were located on the slopes of the easternmost cone ('Eastern Cone'; Fig. 1). Several sites were sampled on the slopes of one of the central cones ('Central Cone'; Fig. 1), including Fe-Mn Crust (288 m), Mid-cone (278 m), Boulder Vent (267 m), and Floc Storm (247 m). The shallowest site—Aquarium (179 m)—was located at the summit of the Central Cone.

Invertebrates and sediments were collected by 'ROPOS' using a suction sampler or by manipulator grabs of fauna and rocks. Organisms from several taxonomic groups were collected at depths of 179 to 461 m (Table 1). Samples were sorted aboard ship, and individuals or separate tissues were wrapped in aluminum foil and stored at −80°C. To obtain stable isotope and fatty acid signatures of particulate organic matter (POM) originating in the photic zone, a 12.4 l seawater sample was taken over the summit of East Diamante, at 100 m depth, collected on a GF/F filter and frozen at −80°C.

Sediment pre-treatment

We obtained sediment samples from Black Forest, Fe-Mn Crust, and Floc Storm, where there were visible deposits of particulate material. Before sediments were analyzed for lipids and stable isotopes, meiofauna (mostly copepods) were removed using a dissecting microscope. In 1 case, we found meiofauna in sufficient numbers for fatty acid and/or stable isotope analysis. Fragments of dead organisms and other recognizable organic debris were left in the samples, and therefore, we refer to these as 'detritus'.

Analysis of benthic primary producers

To assess depth-related trends in chemosynthetic and photosynthetic benthic primary production, and to document faunal assemblages, we used video footage from the R787 and R788 dives. During dive R788, digital images of the benthos (~1 m above bottom) were taken at regular time intervals (~ every 1 to 5 min of travel, every 1 to 2 m depth). Using the best available image for each 1 to 2 m depth category, we quantified the percent cover of microbial

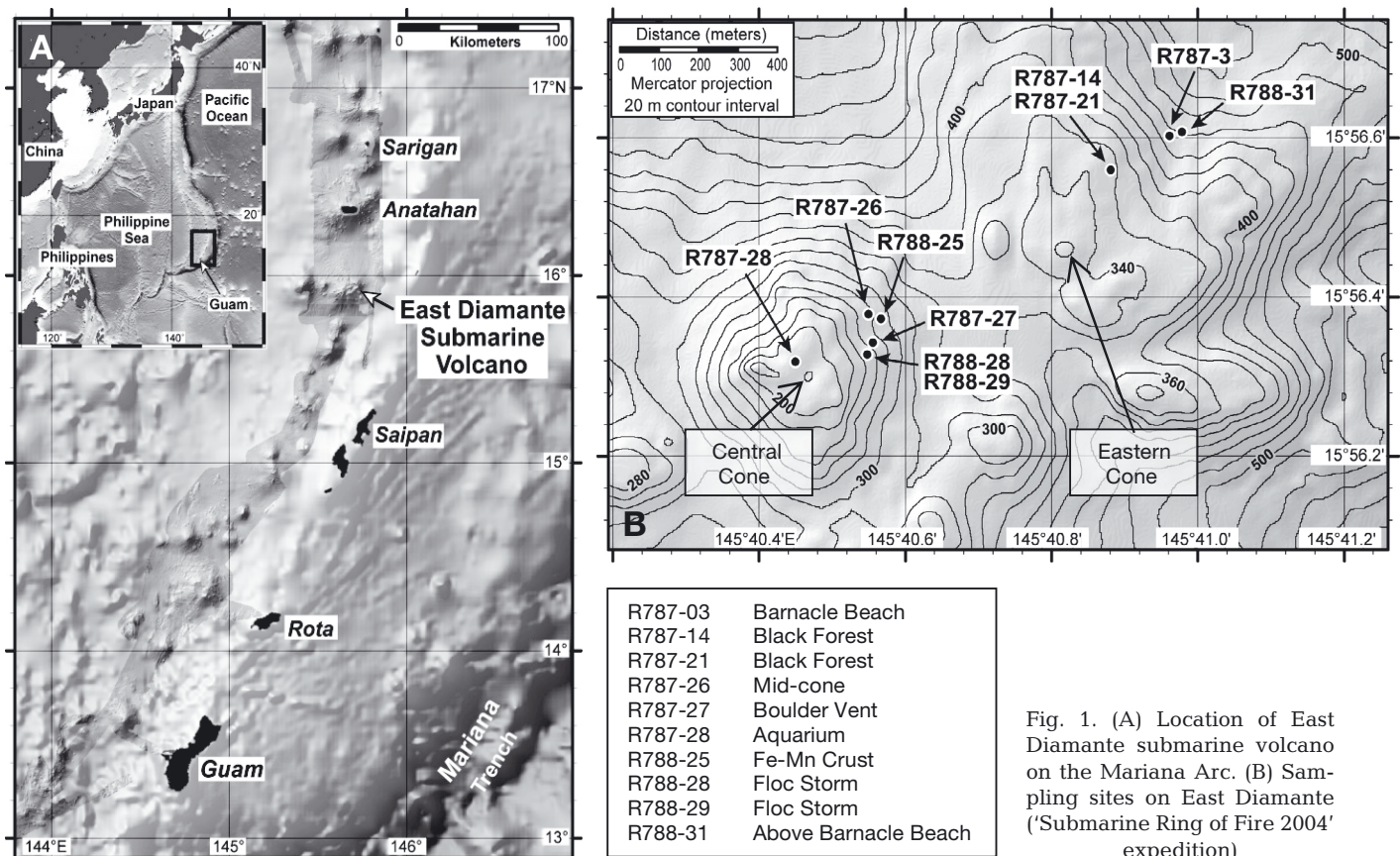| R787-03 | Barnacle Beach |
R787-14	Black Forest
R787-21	Black Forest
R787-26	Mid-cone
R787-27	Boulder Vent
R787-28	Aquarium
R788-25	Fe-Mn Crust
R788-28	Floc Storm
R788-29	Floc Storm
R788-31	Above Barnacle Beach

Fig. 1. (A) Location of East Diamante submarine volcano on the Mariana Arc. (B) Sampling sites on East Diamante ('Submarine Ring of Fire 2004' expedition)

Table 1. Invertebrate sample collection from East Diamante submarine volcano (Mariana Arc) in April 2004, analyzed for stable isotopes (SI) and fatty acids (FA). Unless stated in footnotes, sample sizes refer to numbers of individual animals removed from hard shells or number of tissue pieces

Site	Depth (m)	Dive	Species or sample type	Group	n SI	n FA
Barnacle Beach	457	R787-03	*Neoverruca* n. sp.	Cirripedia	5	4
Barnacle Beach	461	R788-31	*Gandalfus yunohana*	Decapoda	3[a]	0
Black Forest	349	R787-14	*Alviniconcha adamantis*	Gastropoda	3	2
Black Forest	349	R787-21	*Stygiopontius* sp.	Copepoda	5[b]	2[c]
Black Forest	349	R787-21	Amphipod head	Amphipoda	1	0
Fe-Mn Crust	288	R788-25	*Monodonta* sp.	Gastropoda	3	1
Fe-Mn Crust	288	R788-25	Amphipod	Amphipoda	1[d]	0
Fe-Mn Crust	288	R788-25	Polychaete	Polychaeta	1	0
Mid-cone	278	R787-26	*Monodonta* sp.	Gastropoda	3	1
Mid-cone	278	R787-26	Demospongiae tissue	Porifera	1	1
Boulder Vent	267	R787-27	*Shinkailepas* cf. *kaikatensis*	Gastropoda	5	2
Floc Storm	247	R788-28	*S.* cf. *kaikatensis*	Gastropoda	10	3
Floc Storm	247	R788-29	*S.* cf. *kaikatensis*	Gastropoda	10	3
Floc Storm	247	R788-29	*S.* cf. *kaikatensis* egg cases	Gastropoda	2[e]	0
Floc Storm	247	R788-28	Varunid crab	Decapoda	3[f]	1[g]
Aquarium	179	R787-28	*Munidopsis* sp.	Decapoda	1[h]	1[h]
Aquarium	179	R787-28	Basket star arm	Ophiuroidea	0	1
Aquarium	179	R787-28	Demospongiae tissue	Porifera	1	1
Aquarium	179	R787-28	Soft coral tissue	Anthozoa	1	1

[a]Claw or leg muscle from 3 individuals; [b]5 samples of 100 females each; [c]2 samples (first, mixed stages and unknown n; second, n = 2059 individuals [~87 % female]); [d]2 individuals pooled; [e]2 samples of 50 each; [f]Abdominal muscle from 3 individuals; [g]Whole female in shell with eggs attached; [h]1 individual split down the middle — half used for isotopes (abdominal muscle), half for lipids (in shell)

mat and 2 algal taxa (red crusts) with image analysis using SigmascanPro.

Lipid analysis

For the most part, whole animals were used for lipid analysis, but in some cases only tissue subsamples were analyzed (Table 1). Shelled macrofauna (i.e. barnacles, limpets, snails) were removed from their shells before analysis, while the varunid crab and the *Munidopsis* sp. galatheid crab were analyzed with their shells (Table 1). Tissues and sediment aliquots were lyophilized in foil envelopes for 24 h and scraped into Kimax tubes. They were then rehydrated for ~5 min with 0.5 ml water and the foil envelopes rinsed down with chloroform. Samples were ground with a metal rod and lipids were extracted according to Parrish (1999). Before analysis, sediment extracts were passed through copper columns to remove sulfur. Total lipid extracts were derivatized using BF_3-methanol (1.5 h, 85°C), and the resulting fatty acid methyl esters (FAME) were analyzed by flame-ionization detection on an Agilent Model 6890N gas chromatograph (GC) equipped with a DB5 column (30 m ×

0.32 mm × 0.25 µm). Helium was the carrier gas and the column was programmed as follows: 100°C (hold 1 min), 214°C at 4°C min^{-1}, 216°C at 0.5°C min^{-1}, 219°C at 4°C min^{-1}, 223°C (hold 3 min) at 0.5°C min^{-1}, 270°C at 30°C min^{-1}, 315°C (hold 10 min) at 1.5°C min^{-1}. Most fatty acids were identified following Ackman (1986), using the commercial standards '37-Component', 'PUFA No. 1,' and 'Bacterial Acid Methyl Esters' (Supelco, Sigma-Aldrich). After satisfactory GC chromatograms were obtained, several extracts of limpet FAME were pooled to obtain sufficient material for further fatty acid characterization. The resulting identifications were then applied to samples of other organisms by comparison of retention times. Pooled limpets were characterized using pentafluorobenzyl (PFB; Pond & Ward 2011) and picolinyl esters (Christie 2003). FAME were first separated into saturated fatty acids (SFA; no double bonds), monounsaturated fatty acids (MUFA; 1 double bond), dienes (2 double bonds), and polyunsaturated fatty acids (PUFA; 3 or more double bonds) by argentation high-performance thin-layer chromatography (Wilson & Sargent 1992). The PFB and picolinyl esters were analyzed on a Thermo Finnegan GC/MS equipped with a wax column (Restek Stabilwax column, 30 m × 0.25 mm × 0.25 µm).

Stable isotope analysis

In sufficiently large organisms (i.e. crabs and *Munidopsis* sp.), muscle tissue was used for the stable isotope analysis; otherwise whole animals were analyzed (refer to Table 1 for details). Shelled macrofauna were removed from their shells before analysis, and when possible, intestines were discarded. Tissue and filter samples were acidified for 1 min with 0.1 N HCl in glass vials, rinsed once with Milli-Q water, and dried at 55°C for 24 h. Known amounts of dried material were then transferred to tin capsules. When only very small amounts of material were available (e.g. meiofauna), organisms were acidified directly in thick tin capsules, resistant to HCl, and then dried. Carbon:nitrogen ratios of dried sediments were measured before determining isotopic compositions to optimize sample size for mass spectrometry. Stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios were measured using a Micromass Isoprime isotope ratio mass spectrometer, in line with a Carlo Erba C/N element analyzer. Stable isotopic compositions are reported relative to Vienna Pee-Dee Belemnite and atmospheric nitrogen as: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$ (‰), where X is ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Multivariate analysis

We used principal components analysis (PCA) to determine the degree to which vent-obligate invertebrates on East Diamante utilized photosynthetic material. PCA was performed on the invertebrate fatty acid compositional data (% values) and sampling depth (m) using Statistica 6.0. Raw data were converted to *z*-scores before analysis to standardize the weighting of variables measured in different units (Legendre & Legendre 2012). Variables with missing values in >30% of cases were excluded from the analysis.

RESULTS

Biological characteristics of sampling sites

The Eastern Cone hosted 2 active hydrothermal sites (Fig. 1). Barnacle Beach (457–461 m) was a small (2–3 m diameter), well-defined area of diffuse venting through dacite lava boulders covered with barnacles and microbial mats. The hydrothermal vent barnacle *Neoverruca* n. sp. dominated the macrofauna and co-occurred with the arc crab *Gandalfus yunohana*. Further upslope, Black Forest (349 m) is the shallowest actively forming hydrothermal sulfide deposit discovered to date, and several active grey-black smoker chimneys were venting fluids up to 242°C (Embley et al. 2007). Microbial mats, *G. yunohana*, and *Neoverruca* n. sp. were present at the base of chimneys, while *Alviniconcha adamantis* (n. sp.; Johnson et al. 2014) snails clustered around diffuse flow. Butterflyfish (Chaetodontidae) were observed swimming through the chimney field. A copepod swarm (~10 000 ind.), apparently composed entirely of *Stygiopontius* sp., was collected in a sediment sample from the base of a chimney. The sediments at Black Forest consisted of grey silt-like particles and disintegrated sulfide.

The Central Cone was characterized by widespread diffuse venting through pebbles, cobbles, and even between dacite boulders, providing habitat for snail/sponge and limpet/crab assemblages and microbial mats. At Fe-Mn Crust (288 m), there was minor venting through talus coated with sponges, microbial mats, *Shinkailepas* cf. *kaikatensis* limpets, and *Monodonta* sp. snails. Butterflyfish were also observed here. The sediment sample was characterized by reddish particles and contained abundant meiofauna (i.e. 6+ species of foraminifera, halacarids, ostracods, copepods, polychaetes, nematodes) and small macrofauna (i.e. ophiuroids, amphipods, snails, mussels, and gastropod egg cases). Boulder Vent (267 m) was characterized by high-volume discharge of shimmering water, more focused flows, and sub-meter-scale boulders. The steeply sloping blocky talus terrain at this site was coated, often completely, by white microbial mats, and occasional fish were present (Serranidae). On some boulders, the conical limpet *S.* cf. *kaikatensis* was a biomass dominant and was associated with *G. yunohana*. At Floc Storm (247 m), located on a boulder-strewn slope, abundant snow-like floc particles were being advected upslope by strong currents. The limpet *S.* cf. *kaikatensis* was very abundant and was found in association with a small varunid crab. In the glassy sediments, limpet egg cases and trochophores were abundant, and meiofauna included nematodes, foraminifera, siphonotomatoid and harpacticoid copepods, ostracods, snails, halacarids, and polychaetes.

The 195 m depth contour marked the appearance of perceptible ambient light and the beginning of an extraordinary transition from a chemosynthetic productivity regime to one dominated by photosynthesis (see below). At this depth, visible shimmering water indicated widespread hydrothermal venting. Further upslope, no signs of venting were present near the

summit of the Central Cone where a luxuriant suspension feeder community, dominated by soft corals, was discovered (Aquarium; 179 m). Several species of fish were abundant at Aquarium, including *Myripristis chryseres*, *Cephalopholis miniata*, *C. urodeta*, *C. sexmaculata*, *Epinephelus* sp., and *Coradion chrysozonus*, together with damselfish (Pomacentridae), sea urchins, serpulid polychaetes, coralline algae, sponges, and scalpellid barnacles. Jacks (Carangidae), tuna (Scombridae), and a grey shark (*Carcharinus amblyrhynchos*) were swimming near and above the summit.

Benthic primary producers

A thick microbial mat was present throughout much of the dive transects on East Diamante, and completely covered the substratum at depths >200 m (Fig. 2). Mat cover decreased progressively at shallower depths until the mat was no longer visible in images taken above 185 m; the upper limit for microbial mat on East Diamante was estimated to be 188 m. Two morphotypes of coralline algae showed the reverse pattern. They were present in low abundance, overgrown by microbial mat at depths >205 m, and their coverage increased between 200 and 195 m, remaining relatively constant at ~20–40% at shallower depths (Fig. 2).

Fatty acid composition

At hydrothermal vents, varying proportions of particular fatty acids can be used to study mixed diets in invertebrates. Useful bacterial biomarkers are ω4 and ω6 dienes and ω7 MUFA (e.g. 16:1ω7, 18:1ω7), while 20- and 22-carbon PUFA (e.g. 20:4ω6, 20:5ω3, 22:6ω3) can indicate photosynthetic food sources (Pond et al. 1997a,b, Stevens et al. 2008). Although not always present at high concentrations, non-methylene interrupted dienes (NMID) and other MUFA (e.g. 20:1ω7) are commonly encountered in vent invertebrates whose primary source of nutrition is bacteria (Pranal et al. 1996, 1997).

The fatty acid composition of invertebrates at East Diamante submarine volcano differed among sampling sites located along the depth transect. *Neoverruca* n. sp. barnacles at the deep Barnacle Beach site had relatively simple fatty acid compositions, and were characterized by large proportions of MUFA (Table 2). Several individual MUFA contributed to this total, but 16:1ω7 and 18:1ω7 were the most abun-
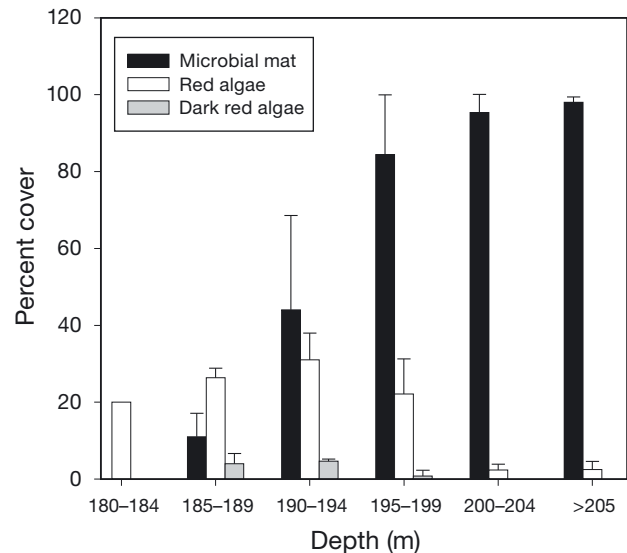

Fig. 2. Distribution of primary producers (bacterial mat, 2 morphotypes of coralline algae) within the photic zone on East Diamante. Error bars represent 1 SD (n = 2–15)

Table 2. Fatty acid composition of barnacles *Neoverruca* n. sp. (n = 4) collected at Barnacle Beach on East Diamante submarine volcano (457 m) during Dive R787-03; SFAs: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; Tr: trace (<0.1% of total). Proportions of non-methylene-interrupted dienes (NMID) are included in summations for both 'ΣDienes' and 'ΣNMID.' Values are expressed as means ± 1 SD

Fatty acid	%
14:1	1.8 ± 0.3
14:0	2.1 ± 0.6
i-15:0	0.1 ± 0.1
ai-15:0	Tr
15:0	Tr
16:1ω7	30.4 ± 2.4
16:0	7.8 ± 0.6
i-17:0	Tr
18:2ω6	13.4 ± 2.9
18:1ω9+ω13	7.4 ± 1.5
18:1ω7	16.0 ± 0.9
18:2ω4	7.0 ± 1.7
18:0	4.2 ± 1.2
20:4ω6	0.8 ± 0.2
20:5ω3	2.1 ± 0.4
20:2Δ5,13	Tr
20:2Δ13,16	0.1 ± 0.1
20:1ω13+ω9	0.7 ± 0.5
20:1ω7	4.8 ± 0.5
22:6ω3	1.1 ± 0.8
22:4	0.1 ± 0.1
22:5ω3	0.1 ± 0.1
ΣSFAs	14.2 ± 1.1
ΣMUFA	61.0 ± 2.4
ΣDienes	20.5 ± 2.4
ΣNMID	0.1 ± 0.2
ΣPUFA	4.2 + 1.3

Table 3. Fatty acid composition (%) of invertebrates at Black Forest on East Diamante submarine volcano (349 m). Abbreviations as in Table 2; dive codes as in Table 1

Fatty acid	*Alviniconcha adamantis* foot R787-14	*A. adamantis* foot R787-14	*Stygiopontius* sp. R787-21	*Stygiopontius* sp. R787-21
14:1				0.2
14:0	1.2	1.6	1.0	1.1
i-15:0			0.2	0.3
ai-15:0				0.1
15:0	0.2	0.1	0.2	0.2
16:1ω7	7.6	0.8	12.1	13.5
16:0	11.3	7.3	15.2	14.1
i-17:0			0.2	0.3
17:0	0.8	0.4	0.6	0.4
18:2?			6.6	6.0
18:2ω6	8.7	10.3	1.0	0.9
18:1ω9+ω13	4.7	6.8	1.6	1.6
18:1ω7	9.8	1.7	24.0	21.4
18:2ω4	0.6			0.7
18:0	7.8	9.3	12.7	9.3
20:4ω6	6.3	8.5	4.9	5.8
20:5ω3	3.8	8.2	2.5	3.4
20:2Δ5,11	2.0	4.7	0.8	1.0
20:2Δ5,13	3.0	10.6	0.7	5.5
20:2Δ13,16	13.5	5.7	4.3	
20:1ω13+ω9	12.9	17.2	3.5	4.5
20:1ω7	3.3	1.8	5.6	5.4
22:6ω3			1.3	2.2
22:4	0.7	1.6		0.3
22:5ω3	1.0	1.8	0.3	0.5
22:2Δ7,13		1.1		0.2
22:2Δ7,15	0.8	0.5	0.7	1.1
ΣSFA	21.4	18.7	30.1	25.8
ΣMUFA	38.3	28.3	46.8	46.6
ΣDienes	28.6	33.0	14.1	15.3
ΣNMID	19.3	22.6	6.5	7.8
ΣPUFA	11.7	20.1	9.0	12.2

dant. The dienes 18:2ω6 and 18:2ω4 were detected in high amounts in *Neoverruca* n. sp. Although several were detected, relative abundances of PUFA were low overall.

At Black Forest, the 2 collected *A. adamantis* snails (foot tissue) had quite different fatty acid profiles (Table 3). The 2 samples differed mostly in the relative abundances of MUFA versus PUFA: the first had higher concentrations of 16:1ω7 and 18:1ω7, and lower relative amounts of all PUFA present. Both samples contained high relative amounts of NMID. The 2 samples of *Stygiopontius* sp. were similar to each other, both being characterized by relatively high levels of 16:1ω7, 16:0, 18:1ω7, and 18:0. Several PUFA were detected, with 20:4ω6 and 20:5ω3 being the most abundant. Generally, invertebrates from Black Forest had moderate PUFA levels and moderate to high concentrations of MUFA.

On the slopes of the Central Cone, fatty acid composition was variable among invertebrate taxa

(Table 4). Most notably, the demosponge contained elevated amounts of 16:1ω7 and 20:1ω7 and no detectable PUFA. The varunid had a unique fatty acid profile within the data set, characterized by moderate levels of 18:1ω9+ω13 and 22:6ω3. Otherwise, the crab had high levels of 16- and 18-carbon SFA and MUFA, and significant amounts of 20:4ω6 and 20:5ω3. The 2 gastropods had dissimilar fatty acid compositions. *Monodonta* sp. snails had elevated levels of 20:4ω6 and NMID relative to the *S.* cf. *kaikatensis* limpets, which were in turn relatively rich in 20:5ω3. Compared to the deeper sites, and excluding the unique case of the MUFA-rich demosponge, invertebrates on the slopes of the Central Cone contained moderate to high amounts of both MUFA and PUFA.

Invertebrates at the summit of the Central Cone, Aquarium (179 m), also exhibited variable fatty acid compositions (Table 5). Again, the demosponge sample was the most distinct, this time containing large

Table 4. Fatty acid composition (%) of invertebrates on the slopes of the Central Cone at East Diamante submarine volcano (247–288 m). Error estimates represent 1 SD. Abbreviations as in Table 2; dive codes as in Table 1

Fatty acid	*Monodonta* sp. R788-25 Fe-Mn Crust	*Monodonta* sp. R787-26 Mid-cone	Demosponge R787-26 Mid-cone	*Shinkailepas* cf. *kaikatensis* (n = 2) R787-27 Boulder Vent	*S.* cf *kaikatensis* (n = 6) R788-28, R788-29 Floc Storm	Varunid crab R788-28 Floc Storm
14:1	0.4			0.3 ± 0.0	0.6 ± 0.4	0.2
14:0	1.0		8.1	0.9 ± 0.1	0.7 ± 0.1	0.8
i-15:0			1.8			0.2
15:0	0.7		0.7	0.5 ± 0.1	0.5 ± 0.1	0.2
16:1ω7	5.2	8.2	41.4	3.5 ± 0.1	4.8 ± 1.4	14.4
16:0	12.8	16.3	17.1	13.8 ± 3.8	13.5 ± 1.1	10.2
i-17:0	1.3			0.1 0.2	0.4 ± 0.2	0.4
17:0	3.0	3.2		0.9 ± 0.0	1.1 ± 0.2	0.6
18:2ω6	2.3	3.7	1.6	3.2 ± 0.3	3.4 ± 0.4	1.8
18:1ω9+ω13	2.1	3.9	2.6	4.3 ± 2.2	6.2 ± 1.4	8.6
18:1ω7	17.8	17.0	5.5	14.7 ± 2.8	17.0 ± 2.5	20.4
18:2ω4	0.4			2.8 ± 1.1	3.3 ± 0.6	1.2
18:0	3.9	4.8	4.9	5.3 ± 0.8	5.3 ± 0.6	6.7
20:4ω6	18.3	14.5		8.3 ± 0.6	6.6 ± 1.1	8.4
20:5ω3	6.4	5.7		16.8 ± 5.0	11.8 ± 1.6	10.6
20:2Δ5,11	0.6	1.3		0.6 ± 0.1	0.5 ± 0.1	0.3
20:2Δ5,13				1.0 ± 0.0	1.8 ± 0.7	0.8
20:2Δ13,16	2.0	2.8		3.6 ± 0.6	3.7 ± 0.9	1.0
20:1ω13+ω9	1.5			12.3 ± 3.0	11.7 ± 1.3	3.3
20:1ω7			16.3	1.6 ± 0.3	2.2 ± 0.6	1.9
22:6ω3						4.1
22:4	5.1			2.2 ± 0.8	1.8 ± 0.2	1.5
22:5ω3	4.6	5.5		1.7 ± 0.4	1.5 ± 0.2	2.2
22:2Δ7,13				0.7 ± 0.4	0.9 ± 0.1	
22:2Δ7,15	10.8	13.2		0.7 ± 0.1	0.7 ± 0.1	
ΣSFA	22.7	24.3	32.5	21.6 ± 4.6	21.5 ± 1.8	19.1
ΣMUFA	26.8	29.1	65.9	36.8 ± 8.3	42.4 ± 4.5	48.9
ΣDienes	16.1	21.0	1.6	12.6 ± 0.5	14.3 ± 1.7	5.2
ΣNMID	13.4	17.2	0.0	6.7 ± 0.9	7.6 ± 0.9	2.1
ΣPUFA	34.4	25.6	0.0	29.0 ± 3.1	21.8 ± 2.6	26.8

relative amounts of 18:2ω6 and very little PUFA. None of the samples contained the high amounts of 16:1ω7 and 18:1ω7 seen at deeper sites. The basket star was characterized by high levels of 20:4ω6 and 20:1ω13+ω9, the galatheid crab *Munidopsis* sp. by 22:6ω3, and the soft coral was exceptionally rich in 20:4ω6. Aside from the sponge, samples from Aquarium were rich in PUFA and had low to moderate MUFA levels.

Samples of detritus from 3 sites (Black Forest, Fe-Mn Crust, Floc Storm) were composed predominantly of SFA (16:0, 18:0) and MUFA (16:1ω7, 18:1ω7), although PUFA were present at Black Forest (20:4ω6, 20:5ω3) and Floc Storm (20:4ω6, 20:5ω3, 22:4, 22:5ω3; Table 6). Water column particulates collected at 100 m over the summit of East Diamante were also dominated by SFA and MUFA and contained a small amount of PUFA (20:5ω3, 22:6ω3) (Table 6).

Principal component 1 (PC1) described ~36% of the total variance in invertebrate fatty acid composition, and PC2, ~16% (Figs. 3 & 4). The PCA identified 4 major groups of samples based on their fatty acid profiles (Fig. 4). The barnacles formed a discrete cluster, with the highest PC1 values, and the projection of the variables within the principal component (PC) space (Fig. 3) showed that proportions of 18:2ω4, ΣMUFA, and sampling depth characterized these samples and separated them from all others. In contrast, 3 samples from Aquarium (soft coral, brittle star, *Munidopsis* sp.) formed a cluster characterized by low PC1 values. This clustering of Aquarium samples was driven by high levels of ΣSFA, 15:0, Σ odd and/or branched fatty acids (OBFAs), and 20:4ω6 (Fig. 3). The demosponge from the Central Cone formed a cluster of its own (low PC2 values) driven largely by the elevated concentrations of 20:1ω7 and 14:0. The remainder of the samples from East Dia-

Table 5. Fatty acid composition (%) of invertebrates at Aquarium on East Diamante submarine volcano (179 m). Abbreviations as in Table 2; dive codes as in Table 1

Fatty acid	Basket star R787-28	Demo-sponge R787-28	*Munidopsis* sp. R787-28	Soft coral R787-28
14:1				0.3
14:0	6.8	1.0	1.4	1.0
i-15:0	4.5	3.2	0.3	0.2
ai-15:0	0.8	2.2		
15:0	2.4	0.9	1.3	0.8
i-16:0	0.6	1.1		0.5
16:1ω7	0.3	3.3	4.5	1.7
16:0	7.1	10.6	18.9	13.5
i-17:0	0.5	2.7	0.5	1.4
17:0	1.2	1.1	3.0	2.5
18:2ω6	0.4	52.3	1.9	1.8
18:1ω9+ω13	1.2	8.8	10.3	2.7
18:1ω7	4.6	3.1	4.8	1.4
18:2ω4	0.4		0.3	0.2
18:0	11.7	6.3	10.2	7.1
20:4ω6	25.6	0.3	12.2	55.4
20:5ω3	9.8	0.1	10.7	4.3
20:2Δ5,13	0.5		0.3	0.6
20:2Δ13,16		1.1		
20:1ω13+ω9	20.8	1.1	0.7	0.4
20:0				0.8
22:6ω3	0.8	0.8	17.2	2.5
22:4			0.6	0.9
22:5ω3			1.0	
ΣSFA	35.6	29.1	35.5	27.6
ΣMUFA	26.9	16.3	20.2	6.6
ΣDienes	1.3	53.4	2.5	2.6
ΣNMID	0.5	1.1	0.3	0.6
ΣPUFA	36.1	1.2	41.8	63.1

Table 6. Fatty acid composition (%) of detritus and particulate matter at East Diamante submarine volcano. Abbreviations as in Table 2; dive codes as in Table 1

Fatty acid	Detritus R787-21 Black Forest	Detritus R788-25 Fe-Mn Crust	Detritus R788-29 Floc Storm	Filter Above summit (100 m)
14:1				0.4
14:0	3.3	4.7	1.1	6.4
i-15:0	1.8	1.7	0.4	0.3
ai-15:0	1.7		0.2	0.5
15:0	1.3	1.5	0.6	1.4
i-16:0	0.8	1.5	0.2	
16:1ω9				2.5
16:1ω7	17.6	7.0	6.8	2.7
16:0	28.6	26.8	18.9	24.4
i-17:0	1.2		0.8	
17:0	1.4	1.4	1.2	1.2
18:2ω6	1.3	2.7	1.8	2.3
18:1ω9+ω13	4.3	10.1	7.1	6.1
18:1ω7	17.5	13.1	25.9	1.0
18:2ω4	1.8	1.4	3.1	
18:0	6.8	26.4	7.8	47.2
20:4ω6	0.5		1.7	
20:5ω3	0.3		2.9	0.5
20:2Δ5,11			0.2	
20:2Δ5,13			0.8	
20:2Δ13,16	2.1	1.7	2.5	
20:1ω13+ω9	3.6		8.8	
20:1ω7	3.0		3.1	
20:0	0.3		0.2	1.8
22:6ω3				1.4
22:4			0.8	
22:5ω3			0.7	
22:2Δ7,13			0.7	
22:2Δ7,15	0.5		0.8	
22:1ω9			0.7	
22:0	0.2		0.2	
SFA	47.6	64.1	31.6	83.2
MUFA	45.9	30.2	52.4	12.7
Dienes	5.7	5.7	9.9	2.3
PUFA	0.8	0.0	6.0	1.9

mante, including all of the limpets, snails, the varunid crab, copepods, and the Aquarium sponge, were part of a cluster centrally located in the PC space (Fig. 4). Samples in this central cluster were characterized by a mixture of biomarkers, and the bulk of the points seemed to be largely driven by ΣPUFA, 20:5ω3, ΣNMID, and 18:1ω7 (Fig. 3).

Stable isotope ratios

The isotopic signal of POM collected at 100 m over the summit of East Diamante (δ15N: 5.2‰, δ13C: −22.1‰) was used as a reference point and potential end member for the sampled invertebrates (Fig. 5). In some cases, the isotopic composition of a detrital sample was also available for comparison. At Barnacle Beach, invertebrates were enriched in 15N and/or 13C, compared to samples at most other sites. More specifically, the arc crabs *G. yunohana* had high δ15N

values, while the barnacles *Neoverruca* n. sp. had some of the highest δ13C values in the data set. The isotopic composition of *G. yunohana* and *Neoverruca* n. sp. diverged significantly from the photic zone POM. At Black Forest, the snails *A. adamantis*, copepods *Stygiopontius* sp., amphipod head, detritus, and photic zone POM formed a cluster of points with moderately enriched 15N and 13C. The δ13C signatures of the invertebrates were similar to both potential food sources.

At Fe-Mn Crust on the slopes of the Central Cone, the snails *Monodonta* sp. were more depleted in 13C than the polychaete, the amphipod, and both the photic zone POM and detritus. All invertebrates had
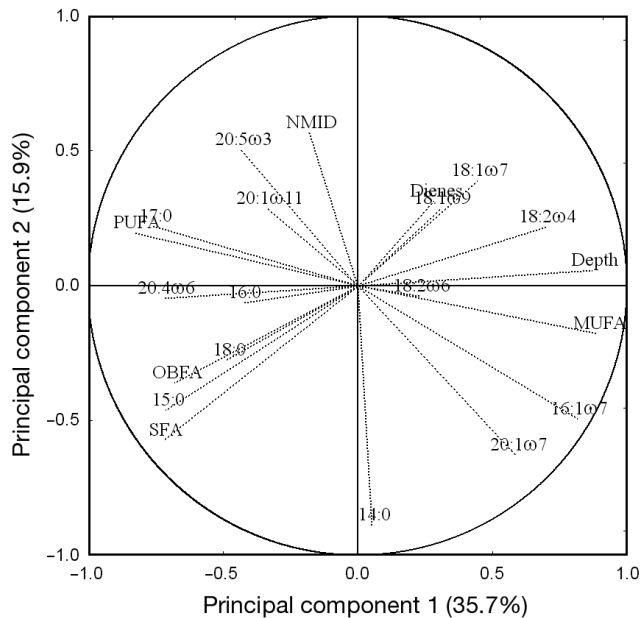
Fig. 3. Results of principal components analysis (PCA) using invertebrate fatty acid composition in samples collected on East Diamante submarine volcano. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OBFA: odd and/or branched fatty acids; NMID: non-methylene-interrupted dienes

similar $\delta^{15}N$ values. The amphipod and the polychaete had $\delta^{13}C$ levels that were similar to both the POM and the detritus. At Mid-cone, the snails *Monodonta* sp. were again significantly depleted in ^{13}C. Conversely, the demosponge was relatively enriched in ^{13}C. Organisms had similar $\delta^{15}N$ values. None of the sampled invertebrates at Mid-cone had carbon isotopic compositions that were similar to the photic zone POM. The limpets *S.* cf. *kaikatensis* at Boulder Vent formed a loose group of points with moderate $\delta^{13}C$ and $\delta^{15}N$ levels, a carbon isotopic composition not dissimilar to that of the photic zone POM. At Floc Storm, samples were tightly clustered, including the detritus and POM. Many of the organisms here (including crabs, limpets, limpet egg cases) had similar $\delta^{13}C$ compositions, centered at approximately −25‰. The $\delta^{13}C$ signatures of some samples were similar to both potential food sources, but except for the crabs, were too depleted in ^{15}N. At Aquarium, invertebrates were relatively enriched in both ^{13}C and ^{15}N. The galatheid crab *Munidopsis* sp. and the soft coral were possibly making use of photic zone POM, but the demosponge was too enriched in ^{15}N for photosynthetic material to have factored in its diet.
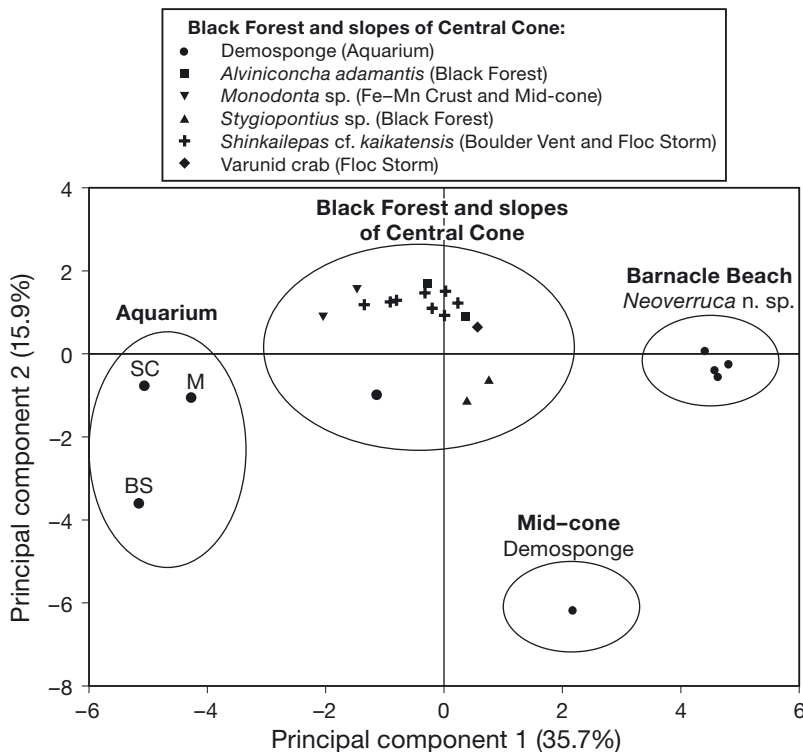

Fig. 4. Results of PCA using invertebrate fatty acid composition in samples collected on East Diamante submarine volcano. Grouping of cases (samples) in the principal components space. SC: soft coral; M: *Munidopsis* sp.; BS: brittle star. Note that the cluster 'Black Forest and slopes of Central Cone' includes 1 sample from Aquarium

DISCUSSION

Clear, depth-related differences in local food webs on East Diamante submarine volcano emerged from the statistical analysis (PCA) of the fatty acid data, and invertebrates were distinguished based on their relative dependence on chemosynthetic and photosynthetic food sources. Invertebrates at Barnacle Beach had a diet based on chemosynthetically derived material, those at Aquarium depended largely on photosynthetic material, and the remaining organisms had mixed diets that included both sources of food. The central cluster of invertebrates with mixed diets contained a large variety of organisms: crabs, limpets, heterotrophic snails, symbiotic snails, copepods, and a sponge, almost all of which were collected at Black Forest and on the slopes of the Central Cone. Therefore, most of the invertebrates sampled above Barnacle Beach on East Diamante, the majority of which were
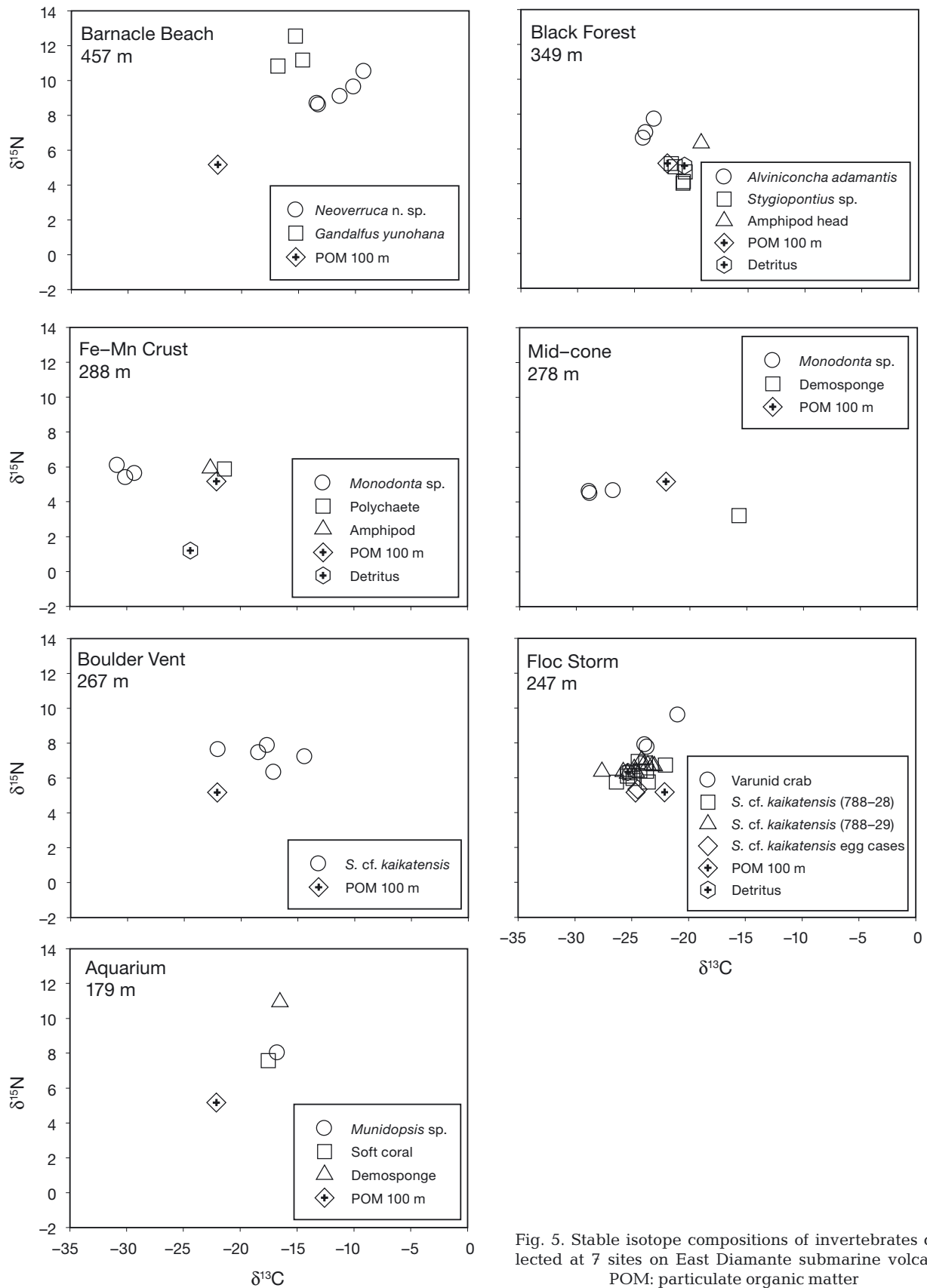
Fig. 5. Stable isotope compositions of invertebrates collected at 7 sites on East Diamante submarine volcano. POM: particulate organic matter

heterotrophic vent-obligate taxa, had a photosynthetic component to their diet. The fatty acid and stable carbon isotope data suggest that planktonic organic carbon is made available to the benthos as marine snow. Fatty acids indicative of phytoplankton (i.e. 20- and 22-carbon PUFA) were found in the water column over the summit of East Diamante and in detritus from both the Eastern and Central Cones. The individual vent sites on East Diamante were populated by different invertebrate taxa, making depth comparisons between individual species impossible. Furthermore, we often lacked sufficient material for replication at individual sites; when we had replicates, the variability was sometimes considerable (e.g. *Alviniconcha adamantis* fatty acids; see below). Although the PCA elucidated broad trophic patterns within the data set, with more complete sampling we may have been able to better differentiate the samples with the mixed diets.

The fatty acid composition of *Neoverruca* n. sp. barnacles at Barnacle Beach suggests a diet rich in bacteria, possibly chemosynthetic bacteria present in vent fluid. Similar fatty acid profiles were found in copepods and amphipods from deep Pacific hydrothermal vents (Limén et al. 2008, Pond et al. 2008) and are generally typical of deep-sea hydrothermal vent fauna (e.g. Ben-Mlih et al. 1992, Pranal et al. 1996, Phleger et al. 2005a, Stevens et al. 2008). The symbiont-containing stalked barnacle *Vulcanolepas osheai* was composed almost entirely of bacterial fatty acids, and no long-chain PUFA were detected (Suzuki et al. 2009). The enriched isotopic signatures of *Gandalfus yunohana* and *Neoverruca* n. sp. indicated a diet based on the 'heavy' carbon often observed at hydrothermal vents, ruling out significant use of photosynthetic material (sensu Goericke et al. 1993). Enriched $\delta^{13}C$ values, typically between −10 and −15‰, have been attributed to the use of the reductive tricarboxylic acid cycle by microbes at the base of the food web, and chemolithoautotrophic bacteria with this heavy isotopic signature are found at hydrothermal vents (Hügler & Sievert 2011).

At Black Forest, the $\delta^{13}C$ levels of *A. adamantis*, *Stygiopontius* sp., and an unidentified amphipod overlapped with those of photic zone POM and detritus, although $\delta^{15}N$ levels in the invertebrates were lower than would be expected if they fed solely on these food sources (sensu Post 2002). Stable isotope and fatty acid signatures in copepods from deeper hydrothermal vents in the Northeast Pacific (>1000 m) do not indicate a similar dietary input from the photic zone (Limén et al. 2008). The fatty acid composition of *A. adamantis* at Black Forest was sim-

ilar to that of *A. hessleri* from deeper vent sites in the Southwestern Pacific (Pranal et al. 1996). It is unclear why the 2 samples of *A. adamantis* foot tissue were dissimilar in their fatty acid composition. Foot tissue was carefully dissected at sea with no apparent contamination (e.g. by gill tissue), and all snails were collected at the same location. POM composition is highly variable over small spatial scales at hydrothermal vents (Levesque et al. 2005, Limén et al. 2007), and the 2 snails may have ingested different food items. Although *A. adamantis* is known to harbor gammaproteobacterial endosymbionts (Johnson et al. 2014), it may supplement its symbiotic mode of nutrition with heterotrophy (references in Pranal et al. 1996).

Generally, the fatty acid and stable isotope composition of invertebrates on the slopes of the Central Cone indicated substantial dietary input of photosynthetic material that presumably originated in the photic zone. While stable isotope ratios and fatty acid biomarkers indicated mixed chemosynthetic/photosynthetic diets in vent limpets and crabs on Volcano-1 (Tonga Arc) (Comeault et al. 2010), the relative importance of photosynthetic material to local invertebrates appeared greater at East Diamante. Compared to limpets and crabs on Volcano-1, varunid crabs and *Shinkailepas* cf. *kaikatensis* on East Diamante were more depleted in ^{13}C and had higher PUFA levels. On Volcano-1, filamentous green algae were found in the digestive tracts of limpets at vent sites within the photic zone (100 m; Comeault et al. 2010), while at East Diamante, marine snow is the likely source of PUFA. The fatty acid composition of *Monodonta* sp. indicated a mixed diet, but stable carbon isotope ratios diverged significantly from those of the photic zone POM. *M. lineata* from non-vent environments may form symbiotic relationships with gammaproteobacteria in the gut (Pratheepa et al. 2014), which could lead to depleted $\delta^{13}C$ signatures if the snails on East Diamante have similar intestinal flora. Compared to *Monodonta* sp. on East Diamante, *M. turbinata*, known from non-vent environments, had substantially lower relative amounts of bacterial fatty acids and higher PUFA levels (Go et al. 2002). *Monodonta* sp. on East Diamante was characterized by high levels of NMID and 20:4ω6, both of which may result from *in vivo* modification of dietary fatty acids (discussed by Pond et al. 2002; see below). The sponges had bacterial fatty acid compositions and stable isotope ratios that diverged significantly from other invertebrates at the same sites. Furthermore, the sponges did not behave as expected in the PCA, with the Mid-cone sponge in a cluster of its own and

the Aquarium sample forming part of the 'mixed diet' central cluster. It therefore appears that sponge diet on East Diamante was not affected by proximity to either hydrothermal venting or the photic zone. The fatty acid composition of these samples may instead reflect the presence of sponge-associated microorganisms (e.g. Ouyang et al. 2010).

As discussed above, there were locally available sources of photosynthetic material at East Diamante and Volcano-1 that could explain the high proportions of PUFA observed in vent invertebrates. However, high levels of 20:4ω6, 20:5ω3, and 22:6ω3 are also found in vent-obligate species whose primary source of nutrition is through symbiosis, and in heterotrophic invertebrates from comparatively deep vents (Pond et al. 2002, 2008, Phleger et al. 2005a,b). Pond et al. (2002) suggested that due to a paucity of preformed PUFA, vent invertebrates may have the ability to desaturate and elongate dietary 18:2ω6 to form 20:4ω6, and 18:3ω3 to form 20:5ω3. The former process has been observed in non-vent echinoderms and corals (Castell et al. 2004, Imbs et al. 2006) and likely explains the significant levels of 20:4ω6 observed in samples from Aquarium. In addition, the important amounts of 20:4ω6 found in many of the invertebrate samples from deeper sites on East Diamante could also be explained in this context, and the dietary source of 18:2ω6 may be vent bacteria. A sample of microbial mat taken from a hydrothermal vent at the neighboring submarine volcano Maug contained a significant relative amount of 18:2ω6 (8%; data not shown); it is possible that similar mats exist on East Diamante. In addition, vent invertebrates may gain access to PUFA through ingestion of microbes that contain these fatty acids. Some deep-sea bacteria produce 20:5ω3 and 22:6ω3 (Nichols 2003), and analogous species may exist at hydrothermal vents. A small amount of 20:5ω3 was detected in the microbial mat sample from Maug (data not shown). The origin of long-chain PUFA in the tissues of hydrothermal vent invertebrates remains inconclusive. Ongoing genomic research and development of analytical techniques may provide the molecular approaches needed to address this issue, by direct study of the enzymes involved in PUFA synthesis and metabolism (reviewed by Monroig et al. 2013).

We have shown that vent-obligate invertebrates incorporate photosynthetic carbon into their diets over a depth range of ~200 m on East Diamante submarine volcano. The invertebrates with mixed diets were a diverse group including small particle feeders (e.g. copepods), grazers (e.g. limpets, snails), and predators/scavengers (e.g. crabs). Photosynthetic material most likely originates in the plankton and arrives at vents as marine snow. At present we cannot quantify amounts or even proportions of chemosynthetic versus photosynthetic food items in invertebrate diets. Furthermore, we do not know whether food selection is important, or whether local vent invertebrates simply ingest whatever is available, in direct proportion to relative abundances. Benthic–pelagic coupling at hydrothermal vents may profoundly affect trophic interactions and community structure. While proximity to vent fluids and temperature gradients regulate the organization of all hydrothermal vent communities, access to photosynthetic standing stocks may have an important structural role at shallow, coastal vents (Tarasov et al. 2005). We have shown that photosynthetic carbon is a prominent component at shallow oceanic arc hydrothermal vent ecosystems.

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