

Chemoautotrophic food availability influences copepod assemblage composition at deep hydrothermal vent sites within sea knoll calderas in the northwestern Pacific

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ABSTRACT: Meiofaunal taxonomic compositions between adjacent hydrothermal vents located in deep-sea volcanic calderas are predicted to differ more than between vents on mid-ocean ridges, as caldera walls are expected to act as barriers to dispersal. However, taxonomic information on hydrothermal vent meiofauna in volcanic calderas is limited. Here, we investigated the spatial variation in benthic copepod taxonomic composition and its association with selected environmental parameters, including food resources, on chimney structures of vent sites in the calderas of 3 neighboring sea knolls (Bayonnaise Knoll, Myojin Knoll, and Myojin-sho Caldera) on the Izu-Ogasawara Arc, northwestern Pacific Ocean. Spatial differences in order-level copepod taxonomic composition were significantly correlated with differences in stable carbon isotopic signatures ($\delta^{13}\text{C}$) of organic matter in the detritus of vent sites; these values reflected the relative contribution of carbon substrates in the vent fluid. The availability of vent chemoautotrophic carbon may have been the primary and a common factor controlling copepod assemblage composition at high taxonomic levels around the hydrothermal vents on these 3 knolls. We also found a bathymetric pattern in the abundance of *Stygiopontius senokuchiae*, a species of copepod in Dirivultidae specific to vents. While this species dominated Bayonnaise Knoll and Myojin-sho Caldera (depth of 740–800 m), their densities were lower in the deeper Myojin Knoll (depth 1250–1320 m). Our results suggest that the caldera wall may not be a significant dispersal barrier for vent copepods; instead, water depth and chemoautotrophic food resource availability are likely the key factors controlling vent copepod distributions.

KEY WORDS: Chemosynthesis · Community composition · Copepod · Deep sea · Dirivultidae · Meiofauna

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INTRODUCTION

Relatively little is known about meiofauna associated with deep-sea hydrothermal vents (Degen et al. 2012, Zeppilli et al. 2015) compared to megafauna taxa specific to these ecosystems, which are dominated by chemoautotrophic primary production (Van Dover et al. 2006). However, the taxonomic

composition of meiofauna has become a focus of attention in recent years (Zeppilli et al. 2018), particularly at vent sites along or near the East Pacific Rise (EPR; Dinet et al. 1988, Tsurumi et al. 2003, Flint et al. 2006, Gollner et al. 2006, 2007, 2010a, 2015a,b, Zekely et al. 2006a,b, Copley et al. 2007, Degen et al. 2012), the Mid-Atlantic Ridge (MAR; Zekely et al. 2006b, Cuvelier et al. 2014, Sarrazin et

al. 2015, Zeppilli et al. 2015, Plum et al. 2017, Baldrighi et al. 2018), and the back-arc spreading centers in the western Pacific (Shirayama 1992, Vanreusel et al. 1997). The compositions of meiofaunal communities have been reported to be markedly similar between vents and adjacent non-vent areas (Vanreusel et al. 2010, Gollner et al. 2015a). This implies that the source of meiofauna communities within each vent field is more commonly invasion from adjacent non-vent-influenced sediments than migration from other hydrothermal vents, interpreted to be due to the small size of meiofauna as well as the lack of a planktonic life stage in many meiofaunal groups (cf. Vanreusel et al. 1997).

Deep-sea hydrothermal vent fields are also distributed in the calderas of seamounts or knolls in island arcs along subduction zones, such as the Izu-Ogasawara Arc in the northwestern Pacific (<http://vents-data.interridge.org/ventfields-osm-map>; accessed 24 November 2018). While vent fields on mid-ocean ridge systems are connected to one another through ridge crests and associated deep currents, those in the calderas of seamounts/knolls are isolated by the high caldera walls that surround them. Despite these geographic barriers between individual calderas, mega- and macrofaunal communities associated with vents on seamounts/knolls on the Izu-Ogasawara Arc have been found to be generally similar among calderas, likely reflecting effective dispersal of planktonic larval stages (Watanabe et al. 2010). In turn, the spatial patterns of vent meiofaunal communities are expected to differ among calderas within the arc, because these small animals have little motility as adults and also rarely exhibit planktonic larval stages (Giere 2009). An exception is the copepod family Dirivultidae (Siphonostomatoida), a typical vent family, which have planktonic nauplii and/or copepodid stages (Ivanenko 1998, Gollner et al. 2015b). As meiofaunal sampling on seamounts is challenging due to the bottom topographies (e.g. steep slope gradient) often combined with hard or rocky seafloor, quantitative meiofaunal data have been lacking except for a small range of seamounts (George & Schminke 2002, George 2013, Zeppilli et al. 2013, 2014). The available information, although limited, implies a high potential of specificity among meiofaunal species on seamounts, even at non-vent sites (George 2013, Zeppilli et al. 2014).

We tested for expected meiofaunal distribution patterns using existing meiofaunal sample sets for both hydrothermal vent sites and adjacent non-vent fields around 3 sea knolls (Myojin Knoll, Bayonnaise Knoll, and Myojin-sho Caldera) in the Izu-Ogasawara Arc

(see Fig. 1). Previous studies of meiofaunal composition at higher taxonomic levels showed that the communities on the surfaces of active vent chimneys were dominated by benthic copepods, while nematodes were the most abundant taxa in the sediments around chimney bases (Kitahashi et al. 2010, Setoguchi et al. 2014, Uejima et al. 2017). Uejima et al. (2017) suggested that copepod dominance is related to high abundances of chemosynthetic food sources around vent chimneys. Hence, detailed information on the taxonomic composition of benthic copepod assemblages, particularly in vent-specific groups such as the Dirivultidae, and their relationship with environmental parameters are crucial for an improved understanding of meiofaunal adaptations to hydrothermal vent ecosystems.

In this study, we focused on spatial variation in copepod community compositions and the population density of *Stygiopontius senokuchiae* Ueyeno, Watanabe & Shimanaga 2018, a vent-specific dirivultid copepod, on active vent chimneys in the 3 knolls. We addressed the following 2 questions: (1) Do copepod taxonomic compositions at vent sites differ among the 3 knolls? (2) Which environmental factors explain copepod taxonomic compositions around hydrothermal vents on these knolls?

Regarding the environmental factors, we considered parameters concerning food resources: total organic carbon (TOC) concentration in detritus, the TOC/total nitrogen (TN) ratio (C/N), and stable carbon isotope ($\delta^{13}\text{C}$) values. Surfaces of active chimneys in the calderas we investigated were covered by aggregations of various sessile mega-macrofaunal species or bacterial mats. These different 'biogenic structures' were also considered as factors that can affect copepod compositions, since some dirivultid species have been reported to show habitat preferences for aggregations of specific vent megafaunal taxa (cf. Gollner et al. 2010b).

Furthermore, we also considered water depth and the height from the chimney base where a sample was taken as potential factors controlling copepod compositions. For some megafauna groups specific to chemosynthesis-based communities, closely related species have different bathymetric distributions within relatively narrow depth ranges (Fujikura et al. 2000). Since there were variations in water depth (740–1300 m) between our investigated vent sites, water depth of the sites was also considered in our analyses. While meiofaunal vertical zonation on sublittoral hard substrata has been studied (Danovaro & Fraschetti 2002), the effects of chimney height on meiofaunal composition has not. This may, however,

affect the colonization of benthic meiofauna from surrounding areas.

All the abovementioned factors (food resource, biogenic structure, water depth, and chimney height) were statistically examined to address how copepod communities are established at hydrothermal vents within the geological setting of calderas in volcanic knolls.

MATERIALS & METHODS

Meiofaunal sampling and sample processing

Samples were collected using the research vessel (RV) 'Natsushima' (cruises NT12-10 in 2012, NT13-09 in 2013, and NT14-06 in 2014; all cruises in April), which visited 3 adjacent knoll-associated calderas with known active hydrothermal vent systems: Bayonnaise Knoll, Myojin Knoll, and Myojin-sho Caldera (Figs. 1 & 2). Meiofaunal samples were collected with the remotely operated vehicle (ROV) 'Hyper-Dolphin'. Detritus was collected from aggregations of the poly-

chaete worm *Paralvinella* spp. on the surfaces of active hydrothermal vent chimneys of Myojin Knoll and Myojin-sho Caldera using a suction sampler (lined with a 30 μ m mesh). These worms are typically distributed in closest proximity to the vent orifice where temperatures are the highest among mega-faunal aggregations (Watanabe & Kojima 2015). At some of the chimneys, we took suction samples from patches of *Neoverruca intermedia*, a vent barnacle that occurs within 10s of cm of the vent orifice. A few water temperature measurements were made with a thermometer at the center of some of the sampling locations, which showed that the *N. intermedia* patches were cooler than the *Paralvinella* spp. patches (Table 1). Since there were no obvious high-density macrofauna aggregations on Bayonnaise Knoll, we collected suction samples from white bacterial mats around the vent orifices of the chimneys. The biogenic substrate, sampling position, water depth, and height above the bottom for each sampling site is summarized in Table 1.

Upon recovery of the samples on the ship, approximately 2 ml of detritus was collected immediately

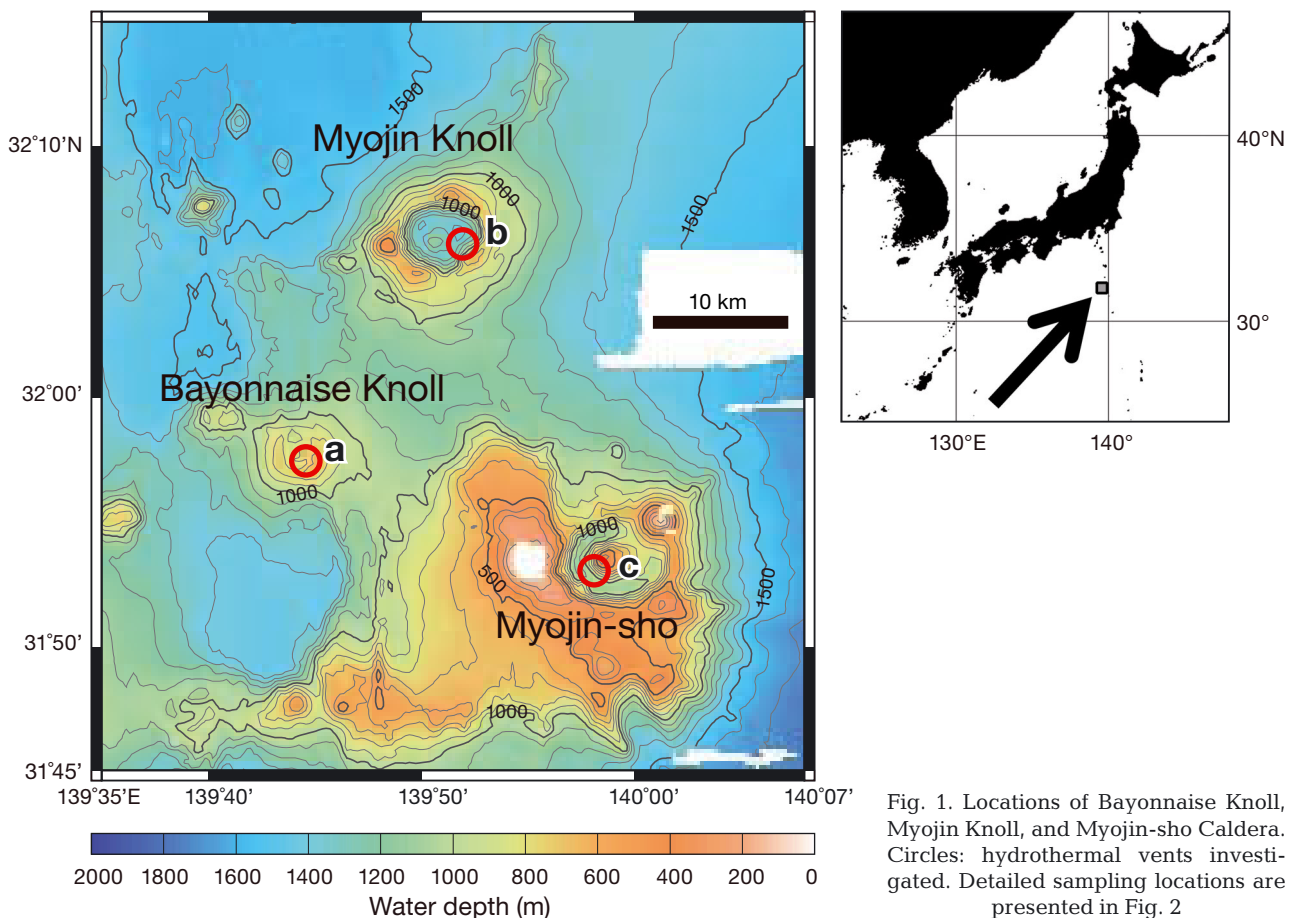


Fig. 1. Locations of Bayonnaise Knoll, Myojin Knoll, and Myojin-sho Caldera. Circles: hydrothermal vents investigated. Detailed sampling locations are presented in Fig. 2

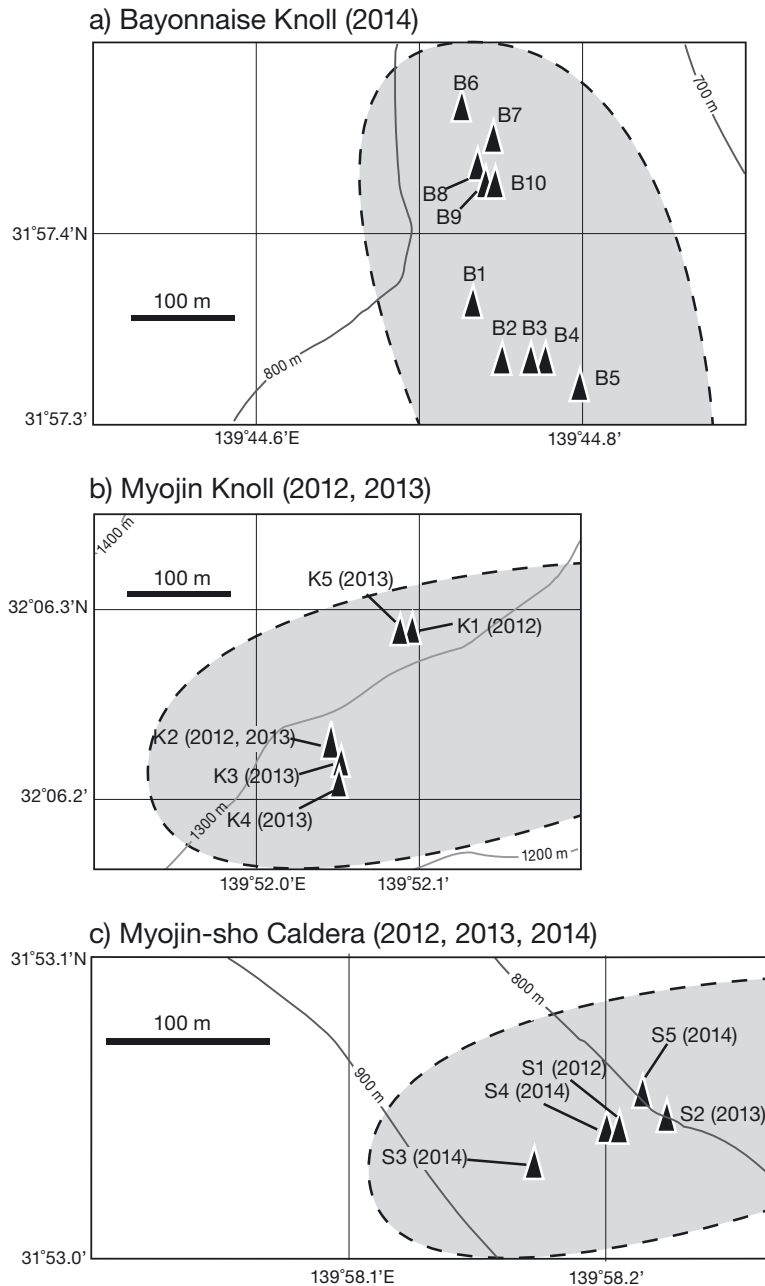


Fig. 2. Detailed sampling locations of active hydrothermal vent chimneys at (a) Bayonnaise Knoll, (b) Myojin Knoll, and (c) Myojin-sho Caldera; sampling chimney identifications and sampling years are also shown (see Table 1 for more details). Sampling years are not shown for sites at Bayonnaise Knoll (a) as all samples were taken in 2014

from each detritus sample (except B4a and K2b because of a simple error and an insufficient volume of detritus for subsampling, respectively; see Table 1) and the 2 ml fractions were frozen without chemical fixation. Those samples were used to measure the concentrations of TOC, TN, the C/N ratios, and $\delta^{13}\text{C}$, to estimate the origins of the organic matter. Details of

the TOC, TN, and $\delta^{13}\text{C}$ analyses are described elsewhere (Ogawa et al. 2010, Setoguchi et al. 2014, H. Nomaki et al. unpubl. data). The total volumes of the initial detritus samples remaining (i.e. after the 2 ml samples had been taken) were measured using a graduated cylinder, and subsequently subdivided into 2 halves using a plankton splitter. One half was preserved in 5% buffered seawater formalin and the other in 99.5% ethanol for analyses of taxonomic composition of meiofauna (used in this study) and their DNA. The total volume of formalin-fixed detritus in each sample is listed in Table S1 in the Supplement at www.int-res.com/articles/suppl/m607p037_supp.pdf. Formalin-fixed samples were then stained with Rose Bengal (final concentration 0.05 g l^{-1}) to help identify live meiofaunal samples.

In the laboratory, the formalin-fixed detritus samples were passed through 1 mm and $63 \mu\text{m}$ mesh sieves, following previous studies of meiofauna taken from hydrothermal vents (Tsurumi et al. 2003, Zekely et al. 2006b, Gollner et al. 2010a, Degen et al. 2012). Detritus remaining on the sieve was resuspended and centrifuged 3 times with colloidal silica (Ludox HS40; Sigma-Aldrich) following the procedures detailed in Danovaro (2010). Supernatants containing meiofauna were transferred to Petri dishes containing 5 mm grids. From the supernatants, the Rose Bengal-stained copepods were sorted, extracted, and counted under a binocular stereo microscope. When the number of copepodids were expected to be more than 1000 by a preliminary check, the supernatants were subdivided using a plankton splitter into fractions of 1/2, 1/4, or 1/8 prior to sorting. The number of extracted copepodids and the fraction size of the subsample for extracting copepodids, which is equal to the proportion of extracted copepodids in total copepodids from each formalin-fixed detritus sample, are shown in Table S1.

After sorting copepodids from the detritus, we randomly selected specimens from these copepodids and identified their copepodid stages until 50 adult individuals had been randomly selected. This was treated as a single sample (or all adults in the whole

Table 1. Sampling locations, sampling codes, dates, biological, and physicochemical parameters. For biogenic substrates, bacteria: bacteria mat; *Paralvinella*: *Paralvinella* patch; *Neoverruca*: *Neoverruca* patch. Height: height above the seafloor as recorded by the remotely operated vehicle's altimeter during fixed-position sampling. Geochemical data on parameters of detritus organic matter are from H. Nomaki et al. (unpubl. data)

Sample ID	Chimney ID	Biogenic substrate	Cruise ID	Date (dd/mm/yyyy)	Depth (m)	Latitude (°N)	Longitude (°E)	Height (m)	Temperature (°C)	Geochemical data
Bayonnaise Knoll										
B1a	B1	Bacteria	NT14-06	13/04/2014	778	31°57.363'	139°44.731'	0.5	23.7	Yes
B2a	B2	Bacteria	NT14-06	13/04/2014	760	31°57.332'	139°44.752'	1.1	–	Yes
B3a	B3	Bacteria	NT14-06	13/04/2014	757	31°57.331'	139°44.765'	1.2	–	Yes
B4a	B4	Bacteria	NT14-06	13/04/2014	757	31°57.331'	139°44.765'	1	–	–
B4b		Bacteria	NT14-06	13/04/2014	757	31°57.331'	139°44.765'	0.8	–	Yes
B5a	B5	Bacteria	NT14-06	13/04/2014	742	31°57.319'	139°44.798'	0.5	–	Yes
B6a	B6	Bacteria	NT14-06	15/04/2014	781	31°57.463'	139°44.726'	1.5	16.4	Yes
B6b		Bacteria	NT14-06	15/04/2014	781	31°57.463'	139°44.726'	1.7	16.4	Yes
B7a	B7	Bacteria	NT14-06	15/04/2014	777	31°57.444'	139°44.744'	1.1	7.4	Yes
B8a	B8	Bacteria	NT14-06	16/04/2014	774	31°57.432'	139°44.736'	1.3	34.2	Yes
B8b		Bacteria	NT14-06	16/04/2014	774	31°57.432'	139°44.736'	1.3	34.2	Yes
B9a	B9	Bacteria	NT14-06	16/04/2014	772	31°57.424	139°44.744'	1.3	150.9	Yes
B10a	B10	Bacteria	NT14-06	16/04/2014	772	31°57.424	139°44.744'	1.3	85.8	Yes
Myojin Knoll										
K1a	K1	<i>Paralvinella</i>	NT12-10	29/04/2012	1321	32°06.286'	139°52.096'	0.7	–	Yes
K1b		<i>Neoverruca</i>	NT12-10	29/04/2012	1321	32°06.286'	139°52.096'	0.7	–	Yes
K2a	K2	<i>Paralvinella</i>	NT12-10	29/04/2012	1249	32°06.214'	139°52.053'	12.7	–	Yes
K2b		<i>Neoverruca</i>	NT12-10	29/04/2012	1249	32°06.214'	139°52.053'	12.7	–	–
K2c		<i>Paralvinella</i>	NT13-09	26/04/2013	1251	32°06.222'	139°52.047'	15.6	–	Yes
K2d		<i>Paralvinella</i>	NT13-09	26/04/2013	1251	32°06.222'	139°52.047'	9.3	–	Yes
K3a	K3	<i>Paralvinella</i>	NT13-09	28/04/2013	1268	32°06.215'	139°52.053'	2.7	–	Yes
K4a	K4	<i>Paralvinella</i>	NT13-09	28/04/2013	1250	32°06.205'	139°52.052'	4	–	Yes
K5a	K5	<i>Paralvinella</i>	NT13-09	26/04/2013	1307	32°06.284'	139°52.092'	1.1	–	Yes
Myojin-sho Caldera										
S1a	S1	<i>Paralvinella</i>	NT12-10	25/04/2012	809	31°53.038'	139°58.210'	1.3	–	Yes
S2a	S2	<i>Paralvinella</i>	NT13-09	24/04/2013	795	31°53.042'	139°58.220'	3.2	–	Yes
S2b		<i>Paralvinella</i>	NT13-09	24/04/2013	795	31°53.042'	139°58.220'	3.4	–	Yes
S2c		<i>Paralvinella</i>	NT13-09	24/04/2013	795	31°53.042'	139°58.220'	4	–	Yes
S2d		<i>Paralvinella</i>	NT13-09	24/04/2013	795	31°53.042'	139°58.220'	3.2	–	Yes
S2e		<i>Paralvinella</i>	NT13-09	24/04/2013	795	31°53.042'	139°58.220'	3.4	–	Yes
S3a	S3	<i>Paralvinella</i>	NT14-06	18/04/2014	850	31°53.028'	139°58.170'	9.4	13.4	Yes
S3b		<i>Neoverruca</i>	NT14-06	18/04/2014	850	31°53.028'	139°58.170'	10.7	6.5	Yes
S4a	S4	<i>Paralvinella</i>	NT14-06	18/04/2014	817	31°53.039'	139°58.201'	1.6	9.6	Yes
S4b		<i>Neoverruca</i>	NT14-06	18/04/2014	817	31°53.039'	139°58.201'	1.5	7	Yes
S5a	S5	<i>Paralvinella</i>	NT14-06	18/04/2014	791	31°53.051'	139°58.212'	7.7	17.9	Yes
S5b		<i>Neoverruca</i>	NT14-06	18/04/2014	791	31°53.051'	139°58.212'	5.6	6.5	Yes

detritus when the numbers were <50) and the adults were then examined under a compound microscope equipped with differential interference optics. The numbers of investigated adults and juvenile copepodids are also listed in Table S1. Taxa were identified to order level using a taxonomic key prepared by Boxshall & Halsey (2004). We investigated only adult copepods due to the difficulty associated with identifying immature copepodites morphologically. The specimen number in a sample (n = 50) was used in our previous studies on meiofaunal composition, and found to be sufficient for evaluat-

ing the overall trend in meiofaunal composition when the total number of investigated specimens is >1000 (cf. Shimanaga et al. 2004, Setoguchi et al. 2014). We also identified specimens of Dirivultidae (Siphonostomatoida), a vent-specific group with a highly characteristic morphology, to genus level using the key prepared by Gollner et al. (2010b). In this group of copepods, we found that all *Stygiopontius* specimens from the 3 calderas belonged to a single species, *Stygiopontius senokuchiae*, based on careful morphological examinations (cf. Uyeno et al. 2018). Although morphological analyses suggested

that the other dirivultids belonged to 2 species (*Chasmato pontius* sp. and *Aphotopontius* sp.), they were identified only to genus level in this study. They will be described in the near future after careful comparisons with the specimens of described species of those genera. A preliminary analysis also indicated that the copepod orders other than Siphonostomatoida have much higher diversities even at family level, and full identification to genus and/or species levels would require at least a few years of taxonomic investigation.

Suction samples from the chimney surface were generally taken from areas of about $20 \times 30 \text{ cm}^2$ in size on common biogenic substrata (e.g. patches of *Paralvinella* spp.), but we encountered difficulties in tracing the sampling areas precisely from video and photographs: the images were not usually captured at appropriate angles to identify the exact sampling points. Furthermore, the variance in the volume of sampled detritus was large among samples, which would have been partly due to the difference in the size of area sampled (Table S1). Thus, the densities of copepods in such samples are expressed as ind. ml^{-1} detritus collected, rather than numbers of individuals per unit area in the present study. We examined the association between environmental factors and population densities of *S. senokuchiae*, but not for other species, because *S. senokuchiae* was the only taxon identified to species level and it was likely the most abundant copepod at the study sites. We also analyzed the densities of the other dirivultids (*Chasmato pontius* + *Aphotopontius*, mainly *Chasmato pontius*) as well as non-Siphonostomatoida copepods. In each case, adult densities of copepod taxa (D) were calculated based on the equation below, where T = the ratio of each taxon per total identified adults, A = the adult ratio in copepodids whose stages were identified, C = number of extracted copepodids, F = fraction size of the subsample for extracting copepodids, and V = total volume of formalin-fixed detritus in each sample (Table S2 in the Supplement).

$$D (\text{ind. ml}^{-1}) = (T \times A \times C) / (F \times V) \quad (1)$$

Statistical analyses

We analyzed the whole data set of order-level compositions (without unidentified specimens) around the knolls using permutational multivariate analyses of variance (PERMANOVA), distance-based linear models (DISTLMs), and distance-based redundancy analyses (dbRDAs) (Clarke & Warwick 2001, Ander-

son et al. 2008). Those analyses were also used for investigating spatial differences in the densities of *S. senokuchiae* (+ the other copepod groups separately). We used the PRIMER 7 software with the add-on package PERMANOVA+ (PRIMER-E) for these analyses.

PERMANOVA was conducted to test the null hypothesis that copepod density and composition did not differ among knolls or biological substrata (i.e. bacterial mats, patches of *Paralvinella* spp., or patches of *N. intermedia*). We used a permutation method that was free of the usual assumption that errors are normally distributed (Anderson et al. 2008). As several samples were collected from the same chimney (Table 1), the chimney from which a sample was taken ('chimney identity' hereafter) was also considered a factor nested in the other factors for all PERMANOVA designs (Anderson et al. 2008). Our sampling design did not cover all investigated habitats around all knolls during all of the sampling periods because of the limited ship time on each cruise; hence, sampling year was not used as a factor in a comprehensive PERMANOVA applied to the whole data set. However, when our considerations were restricted to comparable samples taken from *Paralvinella* patches on Myojin Knoll and Myojin-sho Caldera in 2012 and 2013, a 3-way PERMANOVA (sampling years, sea knolls, and chimneys sampled [as a factor nested in the others]) detected a significant difference in copepod order composition between the knolls, but no significant difference between the sampling years (pseudo- $F = 0.36$, $p = 0.72$). Thus, we concluded that sampling year did not have a significant effect on meiofaunal compositions, and further statistical comparisons were performed without considering year as a factor. In our 2- or 3-way (nested design) PERMANOVAs, we used the 'permutation of residuals under a reduced model' as the permutation method, and Type III sums of squares, which are appropriate for unbalanced samples according to recommendations by Anderson et al. (2008).

To prevent distortions caused by the contributions of the absolute values of densities of some samples that were orders of magnitude larger than others, we used standardized values of copepod abundance for each sample (%) and log-transformed values ($\log_{10}[x + 1]$) for analyses of copepod taxonomic compositions and densities, respectively (Clarke & Warwick 2001). The Bray-Curtis index and Euclidean distance (ED) were used as (dis)similarity indices in the analyses of copepod taxonomic composition and density, respectively. These indices were also calculated using PRIMER 7.

DISTLMs were used to test the multivariate null hypothesis that there is no relationship between meiofaunal parameters and environmental factors (TOC concentration in detritus, C/N ratio, $\delta^{13}\text{C}$ values, water depth, sampling heights above the seafloor [altitude]). DISTLMs were used to determine best-fit models and to partition variance in the response data (meiofaunal density or taxonomic composition) to assign proportional effects to the measured environmental factors (Anderson et al. 2008). Values of the environmental factors were not transformed, as PERMANOVA+ automatically normalizes each environmental factor when running DISTLM (Anderson et al. 2008). TN was found to be highly correlated with TOC ($r > 0.95$) and was excluded from the analyses (Anderson et al. 2008). The forward selection procedure was used to determine the best combination of predictor variables for explaining variation in meiofaunal data and to partition the variation explained by the selected predictor variables based on the selection criterion (Akaike's information criterion [AIC], in this study). DISTLM was used to perform partitioning, test hypotheses, and build models. dbRDA was used to ordinate fitted values from the models selected by DISTLM and to show gradient in the copepod composition among samples that can be modeled by the selected environment factors (Anderson et al. 2008). dbRDA is equivalent to carrying out a traditional RDA if Euclidean distance is used for a dissimilarity index, but any resemblance measure can be used as the basis of dbRDA as well as DISTLM (Anderson et al. 2008).

RESULTS

Community composition of benthic copepods on vent chimneys

Harpacticoida was the most abundant taxon in bacterial mats on the chimneys of Bayonnaise Knoll (88% of investigated adults in each sample, on average; Fig. 3, Table S1), in patches of *Neoverruca intermedia* on Myojin Knoll (ca. 86%), and in patches of *N. intermedia* in Myojin-sho Caldera (ca. 76%). The relative abundances of harpacticoids were lower (42%) in *Paralvinella* spp. patches on Myojin Knoll, where individuals belonging to Cyclopoida and Siphonostomatoida (mainly Dirivultidae) occurred at higher frequencies (Fig. 3). In dirivultids, individuals of *Stygiopontius senokuchiae* were found on all knolls (Bayonnaise, Myojin, and Myojin-sho), and were particularly abundant in patches of *Paralvinella* spp. in Myojin-sho Caldera (79%, on average) (Fig. 3).

When differences between biological substrata were ignored due to one or more biological substrata (i.e. *Paralvinella* spp. patch, *N. intermedia* patch, and bacterial mat) being not sampled consistently across all knolls, pairwise 2-way PERMANOVAs (with chimney identity a nested factor) detected significant differences in order-level composition of copepods (Siphonostomatoida, Cyclopoida, Calanoida, Harpacticoida) between Bayonnaise Knoll and each of the other 2 knolls ($p < 0.01$ for both), although no significance was detected between Myojin Knoll and Myojin-sho Caldera. When analyses were restricted to samples collected from Myojin Knoll and Myojin-sho Caldera, a 3-way PERMANOVA (taking into account the differences between the knolls and the bio-

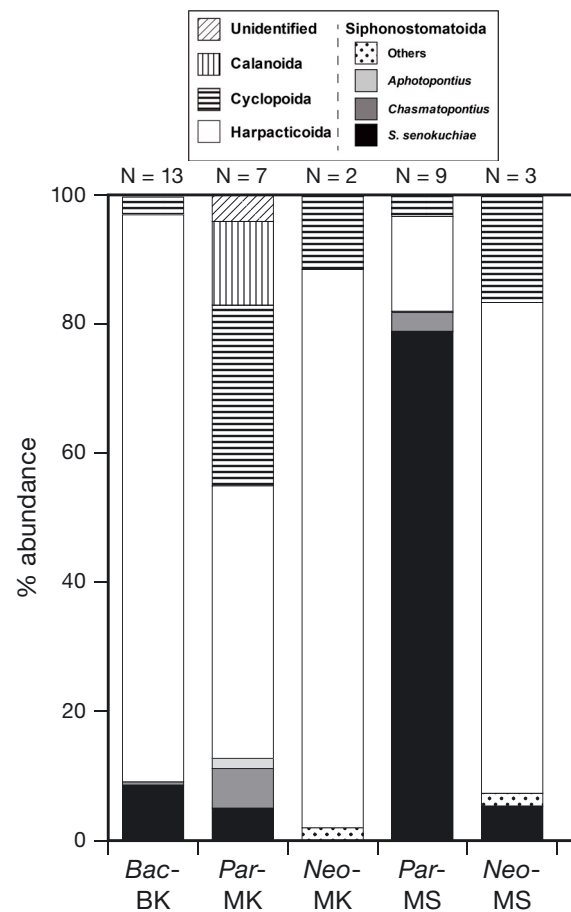


Fig. 3. Mean relative abundances of copepod groups from different biogenic substrata on chimneys in the 3 calderas (*S. senokuchiae*: *Stygiopontius senokuchiae*). N: sample size for each category. Bac-BK: bacterial mats on chimneys at Bayonnaise Knoll; Par-MK and Neo-MK: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin Knoll; Par-MS and Neo-MS: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin-sho Caldera

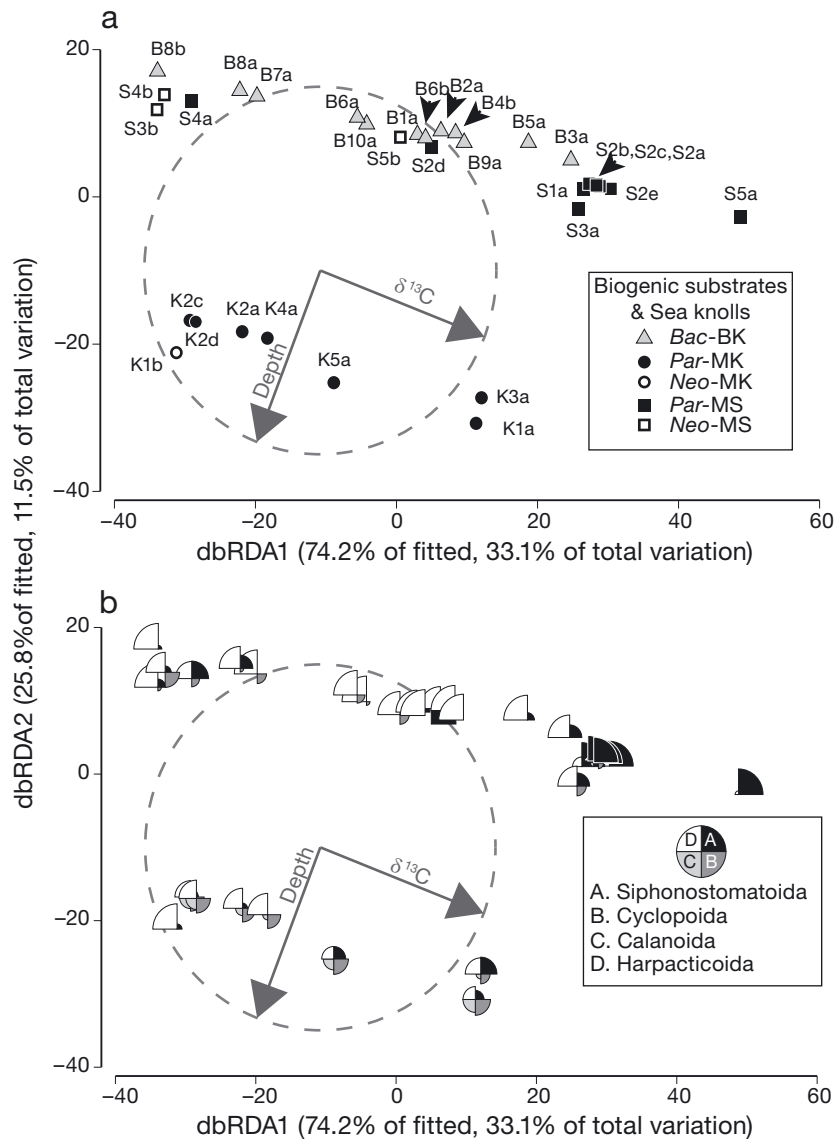


Fig. 4. (a) Distance-based redundancy analyses (dbRDAs) of the entire copepod order-level composition data set according to distance-based linear modeling (DISTLM) with selected environment factors. The percentages of variation in the fitted model and in the total variation in copepod composition explained by each of the dbRDA axes are indicated. The directions and lengths of vector overlays denote the directions and strengths of the relationships between the dbRDA axes and the environmental factors, respectively. Different symbols refer to different habitats where sampling was conducted. *Bac*-BK: bacterial mats on chimneys at Bayonnaise Knoll; *Par*-MK and *Neo*-MK: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin Knoll; *Par*-MS and *Neo*-MS: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin-sho Caldera. Sample codes are arrayed next to the symbols. (b) Segmented bubble plot for the main copepod taxa on the ordination plotted in (a). Differences in size between segmented bubbles indicate relative abundances of the taxa (0–100%)

logical substrata as fixed factors, and chimney identity as the nested factor) detected a significant difference in the order composition of copepods between patches of *Paralvinella* spp. and *N. intermedia* ($p <$

0.01) where the relative abundance of Siphonostomatoida tended to be higher at the former (Fig. 3). On the other hand, no significance was detected between the knolls.

Based on samples for which we had both copepod order-level compositions and environmental data (detritus TOC concentration, C/N ratios, $\delta^{13}\text{C}$ values, water depth, and height of sampling positions on the chimney walls), DISTLM selected only $\delta^{13}\text{C}$ value and water depth as significant factors ($p < 0.001$ for $\delta^{13}\text{C}$; $p < 0.01$ for $\delta^{13}\text{C}$ + depth). The analysis also showed that these 2 factors explained 45% of the total variance in copepod taxonomic compositions among the samples ($\delta^{13}\text{C}$ and depth explained 26 and 19% of the variance, respectively). dbRDA showed that differences in water depth contributed to the difference in order-level composition between Myojin Knoll and the other 2 knolls (Fig. 4a) as well as the proportion of cyclopoids and calanoids (expressed by the sizes of dark gray and light gray segments of bubble plots in Fig. 4b, respectively) tended to be greater on Myojin Knoll. When the analysis was restricted to either only Myojin Knoll samples or Bayonnaise Knoll and Myojin-sho Caldera (2 knolls with similar water depths) combined, spatial differences among samples in copepod order composition were largest along the gradient in the $\delta^{13}\text{C}$ values in each group (Fig. 4a). dbRDA axis 1 was most strongly and positively correlated with $\delta^{13}\text{C}$ values; water depth was negatively correlated with axis 2. The relative abundances of harpacticoids in detritus samples (white segments of bubble plots in Fig. 4b) were negatively correlated with $\delta^{13}\text{C}$ values of those detritus at all calderas, but the ratios of siphonostomatoids in the composition showed a tendency to increase with increasing $\delta^{13}\text{C}$ values, especially in Bayonnaise Knoll and Myojin-sho Caldera (black segments of bubble plots in Fig. 4b).

Densities of *S. senokuchiae* and other copepods, and their associations with environmental factors

When differences in biogenic substrata between samples were ignored, pairwise 2-way PERMANOVAs (with chimney identity as a nested factor) indicated that the log-transformed densities of *S. senokuchiae* at Myojin-sho Caldera were significantly higher than those at Myojin Knoll ($p = 0.0168$) (Fig. 5, Table S2). At Myojin-sho Caldera, the mean density of *S. senokuchiae* in *Paralvinella* spp. patches ($56.5 \text{ ind. ml}^{-1}$) was an order of magnitude higher than that in *N. intermedia* patches (1.9 ind. ml^{-1}), but the difference was not statistically significant because of large within-substratum variance (Fig. 5). *Stygiopontius* was also observed at *Paralvinella* spp. patches at Myojin Knoll, but no specimens were found in *N. intermedia* patches at the same knoll (Fig. 5), although the small sample size ($n = 9$) precluded inferential statistical analyses.

Among the environmental factors considered in this study, DISTLM selected C/N ratio of organic matter in the detritus and water depth as significant factors, each of which explained ca. 19% of the total variance in log-transformed *S. senokuchiae* densities (Table 2). Scatter plots suggest that these factors were negatively

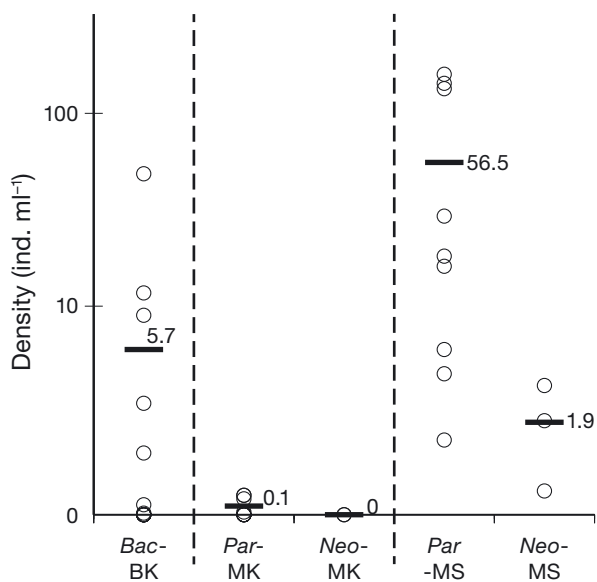


Fig. 5. Densities of *Stygiopontius senokuchiae* on different biogenic substrata on hydrothermal chimneys in the 3 calderas. Bars and adjacent numbers refer to mean densities among samples from the same categories. Bac-BK: bacterial mats on chimneys at Bayonnaise Knoll; Par-MK and Neo-MK: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin Knoll; Par-MS and Neo-MS: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin-sho Caldera

associated with *S. senokuchiae* density (Fig. 6a,b). Higher C/N ratios and lower *S. senokuchiae* densities at Bayonnaise Knoll seemed to contribute to the observed associations. DISTLM also selected the $\delta^{13}\text{C}$ value as a third factor, but the effect was not statistically significant (Table 2). TOC and height above bottom were not selected by DISTLM as explanatory factors for the densities of this species (cf. Fig. 6c,d).

All Myojin Knoll samples were collected from deeper water depths than those from the other 2 calderas (Table 1), and they exhibited lower densities of *S. senokuchiae* regardless of $\delta^{13}\text{C}$ values (filled and opened circles in Fig. 7a). When deeper samples from Myojin Knoll were ignored, the analyses detected a significant and positive association between $\delta^{13}\text{C}$ values and densities of *S. senokuchiae* (marginal test, $p = 0.0184$).

The densities of other Dirivultidae (*Chasmato pontius* + *Aphotopontius*) were low at vent sites in all calderas (average: $<1 \text{ ind. ml}^{-1}$; Table S2 in the Supplement), and no significant pairwise differences were detected in the log-transformed densities among the knolls (pairwise 2-way PERMANOVAs, using chimney identity as a nested factor). The densities were particularly low where $\delta^{13}\text{C}$ values were low ($<-18\%$, Fig. 7b). DISTLM selected only $\delta^{13}\text{C}$ value as a factor explaining the variance in densities,

Table 2. Distance-based linear model (DISTLM) analyses of copepod taxon densities. Variables: selected as predictors in the model; C/N: total organic carbon/total nitrogen ratio; TOC: total organic carbon. Significant variables or pairs of variables and their p-values are shown in **bold**. Var. (%): percentage of the total variation explained by the factor; cumulative (%): percentage explained by the added factors. Other Dirivultidae: summed densities of other genera of Dirivultidae (*Chasmato-pontius* + *Aphotopontius*). ns: not significant

Variables	Pseudo-F	p-value	Var. (%)	Cumulative (%)
<i>Stygiopontius senokuchiae</i>				
+Depth	6.8	0.0124	18.6	18.6
+C/N	8.7	0.0075	18.7	37.3
+ $\delta^{13}\text{C}$	1.9	ns	4.0	41.3
Other Dirivultidae				
+ $\delta^{13}\text{C}$	3.7	ns	10.9	10.9
Cyclopoida				
+ $\delta^{13}\text{C}$	2.7	ns	8.2	8.2
+C/N	4.3	0.0484	12.0	20.1
Calanoida				
+ $\delta^{13}\text{C}$	2.8	ns	8.4	8.4
Harpacticoida				
+ $\delta^{13}\text{C}$	6.1	0.0195	16.9	16.9
+TOC	3.3	ns	8.6	25.5
+Height	2.9	ns	7.0	32.5

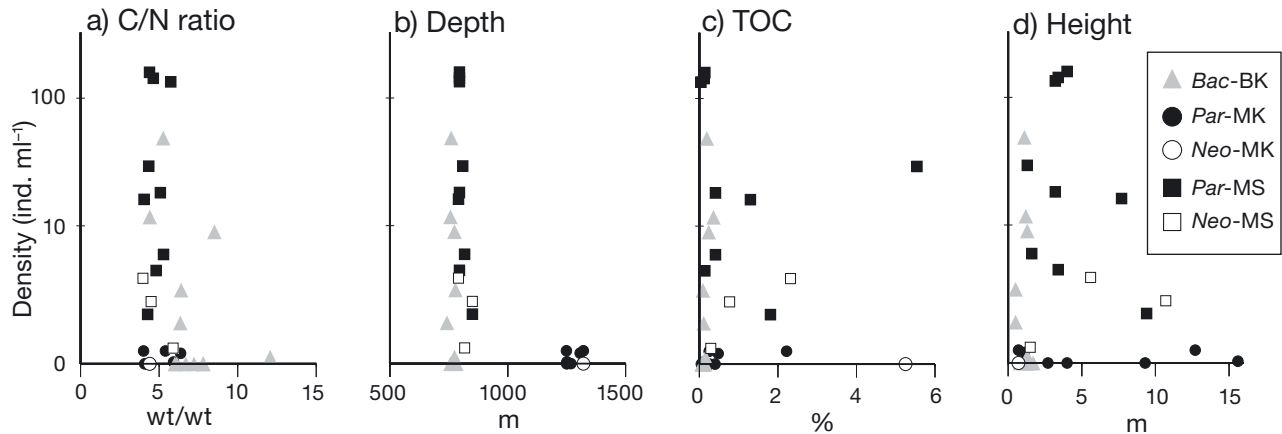


Fig. 6. Plots of the densities of adult *Stygiopontius senokuchiae* against environmental parameters. (a) Total organic carbon/total nitrogen ratio (C/N); (b) water depth at sampling sites; (c) concentration of total organic carbon (TOC); and (d) height of the sampling point above the seafloor. *Bac-BK*: bacterial mats on chimneys at Bayonnaise Knoll; *Par-MK* and *Neo-MK*: patches of *Paralvinella* spp. and *Neoverruca intermedia* respectively, on chimneys at Myojin Knoll; *Par-MS* and *Neo-MS*: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin-sho Caldera

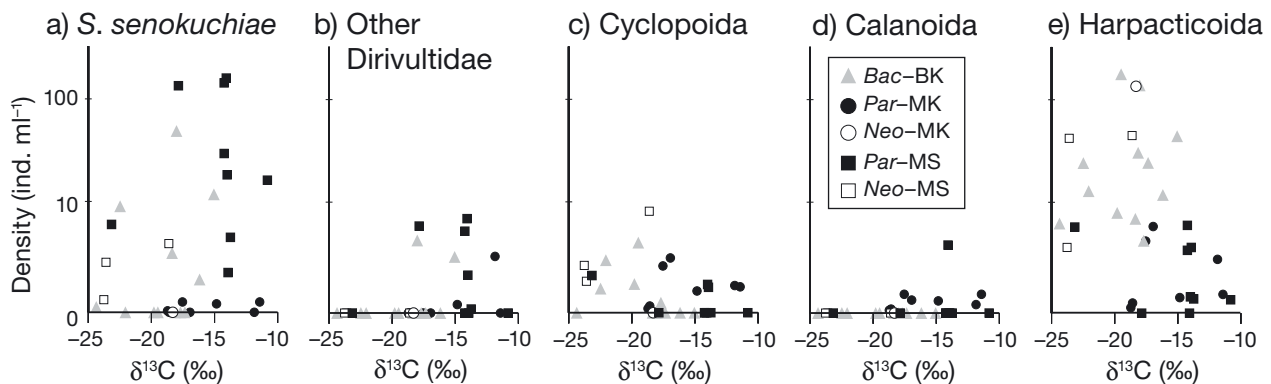


Fig. 7. Plots of densities of (a) *Stygiopontius senokuchiae*, (b) other genera of Dirivultidae (*Chasmatopontius* + *Aphotopontius*), (c) Cyclopoida, (d) Calanoida, and (e) Harpacticoida against stable carbon isotopic compositions ($\delta^{13}\text{C}$) at the sampling sites. *Bac-BK*: bacterial mats on chimneys at Bayonnaise Knoll; *Par-MK* and *Neo-MK*: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin Knoll; *Par-MS* and *Neo-MS*: patches of *Paralvinella* spp. and *Neoverruca intermedia*, respectively, on chimneys at Myojin-sho Caldera

but the effect was not significant (Table 2). The densities of Cyclopoida and Harpacticoida appeared to decrease with increasing $\delta^{13}\text{C}$ values (Fig. 7c,e), but there was no such trend in Calanoida (Fig. 7d). $\delta^{13}\text{C}$ value was selected as an explanatory factor for all of these taxa by DISTLMs (Table 2). $\delta^{13}\text{C}$ value was the only significant factor explaining harpacticoid density variance (17% of total density explained), but it was not a significant explanatory factor for cyclopoid or calanoid density variance when considered alone (Table 2). For cyclopoids, the model selected by DISTLM became significant only when both $\delta^{13}\text{C}$ and C/N were added as its predictor variables (Table 2). Water depth was never selected by the DISTLMs as an explanatory factor for these taxonomic groups other than Dirivultidae.

DISCUSSION

Community composition in relation to carbon content

Studies on stable isotope ratios of meiofauna around hydrothermal vents are still limited compared to works published on macrofauna (Limén et al. 2006, 2007, 2008, Kouris et al. 2010, Gaudron et al. 2012, Portail et al. 2016). Nutritional sources used by vent meiofauna appear to shift from chemoautotrophic microbes to other resources along the gradient from vent orifice outwards to the surrounding non-vent seafloor (Limén et al. 2007). In a previous study, we also found that spatial changes in meiofaunal taxonomic composition at the phylum/class level

(e.g. nematodes, copepods) across the same gradient were significantly correlated with differences in $\delta^{13}\text{C}$ signals in the organic matter within habitats (Uejima et al. 2017). The $\delta^{13}\text{C}$ values of hydrothermal vent chimney surfaces were generally high, up to -10‰ , while those at non-vent sites were closely similar to normal seafloor sediments, i.e. -24 to -22‰ (H. Nomaki et al. unpubl. data).

In the present study, we also found that differences in the order-level composition of copepods were significantly associated with $\delta^{13}\text{C}$ values across various biogenic substrates of the same active vent chimney. Distributional relationships between copepod taxonomic compositions and ^{13}C values were similar among the 3 knolls, even though they were separated from one another by caldera walls. At sites where $\delta^{13}\text{C}$ values were higher (-19 to -11‰) among the examined detritus, i.e. in most of the *Paralvinella* spp. patches, Siphonostomatoida (mostly composed of Dirivultidae) dominated, particularly in Myojin-sho Caldera (Figs. 4 & 7). This implies that the abundance of dirivultids is correlated with vent activity, as reported at mid-ocean ridge vent sites in the eastern Pacific (e.g. Tsurumi et al. 2003, Limén et al. 2007, Gollner et al. 2015a). Both absolute densities and relative abundances of the Harpacticoida, however, decreased with increasing $\delta^{13}\text{C}$ values on all knolls, which implies a weak association between distribution of harpacticoids and organic matter produced by chemoautotrophic microbes that use carbon substrate in vent fluids around *Paralvinella* patches. Similar compositions were found at a vent site on the MAR using artificial substrate introduction, where vent specialists such as dirivultids dominated the copepod fauna on artificial substrata placed at sites of high vent activity (Plum et al. 2017). As temperatures and fluid inputs decreased with increasing distance from vent orifices, non-vent specialists such as harpacticoid copepods increased in abundance (Plum et al. 2017).

Our results are consistent with previous studies on the EPR and the MAR, despite not being based on mid-ocean ridges but on 3 calderas isolated from one another. We showed that $\delta^{13}\text{C}$ signals of organic matter in the detritus, which are indicators of relative contributions of different carbon sources, may be a primary factor in explaining spatial changes in copepod taxonomic compositions on active chimneys across different hydrothermal vent ecosystems. Within the vent fields, 3 major organic carbon sources with characteristically different $\delta^{13}\text{C}$ values have been reported (Yorisue et al. 2012, H. Nomaki et al. unpubl. obs.): organic matter produced by chemoautotrophic microbes in *Paralvinella* spp. colonies with high $\delta^{13}\text{C}$ values (ca.

-11‰); organic matter with relatively low $\delta^{13}\text{C}$ values (ca. -30‰) produced in *Neoverruca intermedia* colonies by chemoautotrophic microbes with diverse carbon-fixation pathways; and organic matter produced by phytoplankton photosynthesis ($\delta^{13}\text{C}$ values of ca. -22‰). In our study, the $\delta^{13}\text{C}$ values of vent chimney detritus ranged from -25 to -10‰ , i.e. a range extending from mixtures of chemoautotrophic organic matter and photosynthetic carbon typifying *N. intermedia* patches to signatures of pure chemoautotrophic organic matter produced in *Paralvinella* spp. patches. The close correlation between $\delta^{13}\text{C}$ values and copepod taxonomic compositions across these different carbon sources implies that the contribution of chemoautotrophic carbon in *Paralvinella* patches, which largely rely on the carbon substrate from the vent fluid (H. Nomaki et al. unpubl. obs.), is among the major determining factors of the copepod taxonomic composition.

Since the activity of chemoautotrophic bacteria in the *Paralvinella* patches in close proximity to the vent orifice is correlated with chemico-physical conditions around vents, $\delta^{13}\text{C}$ values of organic matter and copepod compositions may in fact be controlled simply by chemico-physical conditions in the vent environment, e.g. temperature or sulfide concentration. In the present study, we were not able to include temperature in our overall multivariate analyses due to the limited instances of *in situ* temperature measurements ($n = 14$; Table 1) resulting from limited bottom and ship time. However, we performed an additional DISTLM, using water temperature as an environmental factor, on a subset of the data for which these data were available. This analysis selected $\delta^{13}\text{C}$ and temperature as the first and second explanatory factors, which explained 25 and 11% of the total variation in the order-level composition of copepods, respectively, but neither were statistically significant. This suggests that temperature, which is indicative of the relative composition of the vent fluid and the surrounding bottom water, does not directly explain copepod taxonomic composition. $\delta^{13}\text{C}$ values, which are indicators of the relative contributions of chemoautotrophic organic matter derived from carbon contained in the vent fluid, can explain copepod taxonomic compositions better than water temperature alone.

On the other hand, sulfide concentration in the vent fluid can have a direct effect on $\delta^{13}\text{C}$ values of the detritus because chemoautotrophic microbes derive energy from oxidation of reduced substances such as hydrogen sulfide (H_2S), which are higher in concentration where *in situ* chemosynthetic primary production occur around deep-sea hydrothermal

vents (cf. Gollner et al. 2015a). At vent sites with the highest H_2S concentration, a low diversity of non-dirivultid meiofauna has been reported, probably due to environmental stresses from vent fluids, a major one being the high concentration of reduced substances (Vanreusel et al. 1997, Gollner et al. 2015a). Since we have no available data on the sulfide concentration for our sampling sites, future surveys of small-scale spatial changes in both, the factors concerning sulfides and the quality of organic matter around vents, are required to evaluate their relative contributions to meiofaunal taxonomic composition.

Mechanisms explaining the dominance of Dirivultidae at hydrothermal vents

Most copepod species collected and described from deep-sea hydrothermal vents are members of the family Dirivultidae, a group that occurs only at vent sites (Zeppilli et al. 2018). Dirivultidae is also common among benthic copepod assemblages associated with hydrothermal megafaunal aggregations (e.g. tubeworms, mussels) on hard substrata (Gollner et al. 2010b); for example, this group dominates (~80%) *Paralvinella* patches on the Juan de Fuca Ridge in the eastern Pacific (Tsurumi et al. 2003).

In our study, the relative abundances of dirivultids (*Chasmatopontius* + *Stygiopontius*) were higher in patches of *Paralvinella* spp. than in patches of *Neoverruca intermedia* at both Myojin Knoll and Myojin-sho Caldera. Significant differences in their abundance may not simply reflect a preference for *Paralvinella* spp. as habitat, but rather more likely a preference for the nutritional or environmental conditions where the worms occur, as dirivultids also showed higher densities on bacteria mats at Bayonnaise Knoll. In other regions, most dirivultids have been found to colonize aggregations of more than one mega-macrofaunal species (cf. Gollner et al. 2010b), indicating a low affinity to specific mega-macrofaunal species. Plum et al. (2017) also showed that differences in the types of substratum had weaker effects on copepod colonization and taxonomic composition than the extent of fluid input or water temperature at vent sites.

In the axial summit trough of the EPR, some dirivultids live in a high temperature (somewhere between 14–119°C) in concentrated vent fluid flow (Gollner et al. 2010a). These animals are highly motile, and this may allow them to cope with extremely high spatiotemporal variations in such stressful environments more effectively than other, less-motile copepod groups (Gollner et al. 2015a). Members of Dirivulti-

dae possess hemoglobin with a particularly high oxygen affinity (Hourdez et al. 2000), which may enable them to persist in oxygen-depleted environments near hydrothermal vent orifices.

Mouthpart morphologies and/or stable isotope ratios of dirivultid tissues indicate that most species may be considered primary consumers that graze on chemosynthetic bacterial mats and detritus (Heptner & Ivanenko 2002, Limén et al. 2008). Gollner et al. (2015a) showed that the body sizes of dirivultids exceed those of harpacticoids, and implied that large body sizes and energy demands for reproduction are the primary drivers for this group evolving to become specialized in vent environments. The positive correlation of dirivultid density with increasing $\delta^{13}\text{C}$ in the detritus shown in the present study strongly suggests that effective adaptations to the vent environment and a high demand for chemosynthetic food have driven the dominance of the Dirivultidae at hydrothermal vents, agreeing with previous studies.

Spatial differences in densities of *Stygiopontius senokuchiae*

Densities of *S. senokuchiae* in Myojin-sho Caldera were significantly higher than those on Myojin Knoll, but not between Bayonnaise Knoll and each of the other 2 knolls. Densities of this species were significantly associated with C/N ratios of organic matter in the detritus; these ratios indicate the origin and quality of the organic matter. The dirivultid densities reflected a strongly negative correlation between C/N ratio and $\delta^{13}\text{C}$ signatures, as reported for deep hydrothermal vent sites in other regions (Limén et al. 2007). $\delta^{13}\text{C}$ values were significantly associated with densities of *S. senokuchiae* in the 2 knolls other than Myojin Knoll. However, the relative abundance (and absolute densities) of *S. senokuchiae* in *Paralvinella* spp. patches of Myojin Knoll were lower than the same of Myojin-sho Caldera, although $\delta^{13}\text{C}$ values were similar between the sites (Fig. 7a). These differences in the density of *S. senokuchiae* between Myojin Knoll and other 2 knolls with shallower depths would have caused 'water depth' to be chosen by DISTLM as a further significant factor explaining order-level composition of copepods. Unlike harpacticoid copepods and other meiofaunal taxa such as nematodes, dirivultids such as *Stygiopontius* have planktonic larval stages (Ivanenko 1998). Therefore, caldera walls probably do not act as effective dispersal barriers for *S. senokuchiae*, and the differences in its density requires another explanation.

The *S. senokuchiae* populations sampled in this study were clearly distributed in shallower depths than populations of other congeners, most of which occur at depths of >2000 m (Humes 1987, 1989, 1996, Ivanenko et al. 2006, Gollner et al. 2016). Such differences in depth ranges among taxa may reflect a bathymetric zonation of *S. senokuchiae*, whose lower depth limit may occur at ca. 1500 m. Among megafaunal groups in deep-sea chemosynthesis-based communities in Japanese waters, various species of vesicomid clams have relatively narrow depth distributions (<1000 m; Fujikura et al. 2000). A congener of *S. senokuchiae*, *S. senckenbergi*, has been found on a hydrothermal volcano in the southwestern Pacific at depths (ca. 1600 m; Ivanenko & Ferrari 2013) similar to those sites where we collected *S. senokuchiae*. The volcano on which *S. senckenbergi* was found may be connected to the Izu-Ogasawara Arc through the Mariana volcanic arc.

In summary, the present study provided the first insight into benthic copepod taxonomic compositions, and their relationships with some selected environmental factors, in hydrothermal vent sites within deep-sea knoll calderas. A common trend found was that the spatial differences in order-level copepod taxonomic compositions were correlated with differences in stable carbon isotopic signatures ($\delta^{13}\text{C}$) of organic matter in the detritus at vent sites in all investigated calderas, although the assemblages were spatially separated from one another by caldera walls. This suggests that the availability of carbon derived from chemosynthesis and ultimately the vent fluid may be a common factor controlling copepod assemblage composition (at least at high taxonomic levels) around vents on these knolls. We also found a bathymetric pattern in the density of the dirivultid copepod *S. senokuchiae*, which may reflect the bathymetric zonation exhibited by this species. We cannot, however, rule out the possibility that the low density of *S. senokuchiae* found in the caldera of Myojin Knoll, the deepest of the 3 knolls, may be explained by factors that were not measured in our study, e.g. factors that may not be directly related to depth, such as sulfide concentration. Studies on hydrothermal vent copepod communities in the calderas of the other neighboring seamounts or knolls at depths similar to Myojin Knoll and closer to the Mariana volcanic arc (e.g. Suiyo Seamount, 28° 35' N, 140° 39' E, depth 1300–1400 m; Watanabe et al. 2010) would help clarify these issues.

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