



## Copepod assemblages along a hydrothermal stress gradient at diffuse flow habitats within the ABE vent site (Eastern Lau Spreading Center, Southwest Pacific)

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### ABSTRACT

Copepoda is the most diverse and abundant taxon at deep-sea hydrothermal vents, contributing significantly to the biodiversity and thus is important in maintaining ecosystem functioning. To date we lack knowledge on meiofauna, specifically copepod community dynamics, in the entire region of the Eastern Lau Spreading Center. We explore abundance and diversity of copepods associated with foundation species thriving at distinct physiochemical regimes at the active vent site ABE. Diffuse flow habitats within ABE are dominated by three foundation species: (1) the snail *Alviniconcha hessleri*, exposed to average vent fluid temperatures of  $\sim 24$  °C, (2) the snail *Ifremeria nautilei* at  $\sim 12$  °C, and (3) the mussel *Bathymodiolus septemdirum* in average temperatures of  $\sim 6$  °C. In each habitat, three samples were taken using a quantitative collection device (mussel-pot). Copepod abundance ranged from 1 to 30 ind. per 10 cm<sup>2</sup>, with no significant differences between the three habitats. A total of 18 copepod species were identified. In the *Alviniconcha* habitat, the vent endemic dirivultid copepod *Stygiopontius lauensis* dominated the community, representing >90% of the individuals. Species richness and Shannon diversity were significantly higher in the *Bathymodiolus* habitat, correlated with lower temperatures, lower hydrogen sulfide concentrations and higher oxygen concentrations. This increase in diversity was driven by a community mostly comprised of habitat generalists including genera reported from shallow coastal waters, shallow vents, and peripheral vent environments. This study provides the first quantification of biodiversity and community structure of copepods along a physiochemical stress gradient at a relatively stable Southwest Pacific vent site prospective for deep-sea mining.

### 1. Introduction

The distribution of organisms at deep-sea hydrothermal vents is well known to be associated with their physiological tolerance to extreme conditions of high temperature, high concentrations of reduced chemicals of hydrothermal vent fluid emissions and low oxygen levels. These environmental conditions give way to highly structured meta-communities (Van Dover, 2000; Podowski et al., 2010; Sen et al., 2014; Mullineaux et al., 2018). Hydrothermal vents are volatile, patchily distributed ecosystems scattered across ocean ridges and back-arc basins. Here, disturbance takes the form of volcanic eruptions

occurring at frequencies (decadal to hundreds of thousands of years) that depend on the spreading rate of the plates upon which they sit, ranging from super-fast to ultra-slow (Rubin et al., 2012). Physiochemical conditions that are typical of these systems fluctuate on very small spatial and temporal scales (Childress and Fisher, 1992). Although the organisms found at hydrothermal vents are well adapted to such extreme conditions (Hourdez et al., 2000; Hourdez and Lallier, 2007; Gartman et al., 2011), the rapid changes in vent chemistry constantly test both the physiological and biochemical limits of their tolerance (Waite et al., 2008; Henry et al., 2008; Gollner 2010b). These gradients can therefore be described as stress gradients along which vent

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communities are structured and can be characterized. These communities are dependent on chemosynthetic primary production. In contrast to surrounding waters and benthic environments, chemolithoautotrophic bacteria harness the energy produced by the disequilibrium of proton gradients (Lane et al., 2010) at redox interfaces created by the mixing of ambient oxygenated water and reduced hydrothermal effluent. These bacteria are both free-living and symbiotic, thus able to support unique and intricate food webs within a complex ecosystem (Govenar, 2012).

The ABE hydrothermal vent field includes areas of diffuse hydrothermal venting within the Lau Basin ( $20^{\circ}45'42.8''$  S,  $176^{\circ}11'27.6''$  W) (Fig. 1). Diffuse venting on the seafloor occurs where high-temperature fluids rising from depth become mixed with cold seawater before they exit the seafloor. The mixing of reduced compounds with seawater is higher in diffused flow venting systems, the effluent emerges from the seafloor over a wider area, and is thus lower in temperature ( $2^{\circ}\text{C}$ – $80^{\circ}\text{C}$ ) than focused flow vent systems. The latter exhibit reduced fluids exiting the seafloor from depth having had little or no contact with ambient seawater and the effluent here can reach temperatures of  $400^{\circ}\text{C}$  (Bemis et al., 2012).

The warm sulfide-rich water of diffuse flow hydrothermal vents provides the energy and the basis for a unique ecosystem, where symbiotic megafauna act as foundation species and provide habitat for an

abundant associated fauna (Desbruyères, 1994; Van Dover, 2000; Luther et al., 2012). Typical megafaunal assemblages of the Lau Basin vents include *Alviniconcha hessleri*, a thermophilic provannid snail that has adapted to high concentrations of reduced compounds and dominates at newly formed, hot, sulfidic vents, followed in succession by the less heat-tolerant provannid snail *Ifremeria nautiliei* and eventually the mussel *Bathymodiolus septemdiarium*, that exhibits much lower tolerances to these typical vent conditions, on the low-end of the stress gradient (Podowski et al., 2010; Sen et al., 2014) (Fig. 1). The megafauna here creates a topographically complex substrate relative to their surroundings, providing shelter from predation by mobile organisms (Du Preez et al., 2012).

The study of meiofauna, despite constantly improving methods for molecular taxonomy and molecular methods for estimating biodiversity, is still time-intensive due to the necessary taxonomic work involved (Fontaneto et al., 2015). Studies on meiofauna in extreme environments have, however, gained considerable attention in recent years (see Zepili et al., 2018 for a review). Meiofauna are the dominant size fraction in benthic communities in terms of abundance and diversity, are well known to play important functional roles (Chapman et al., 2018; Schratzberger and Ingels, 2018), and are closely associated with deep-sea megafauna assemblages in many vent ecosystems around the world (Copley et al., 2007; Gollner et al., 2010b). Not only are they

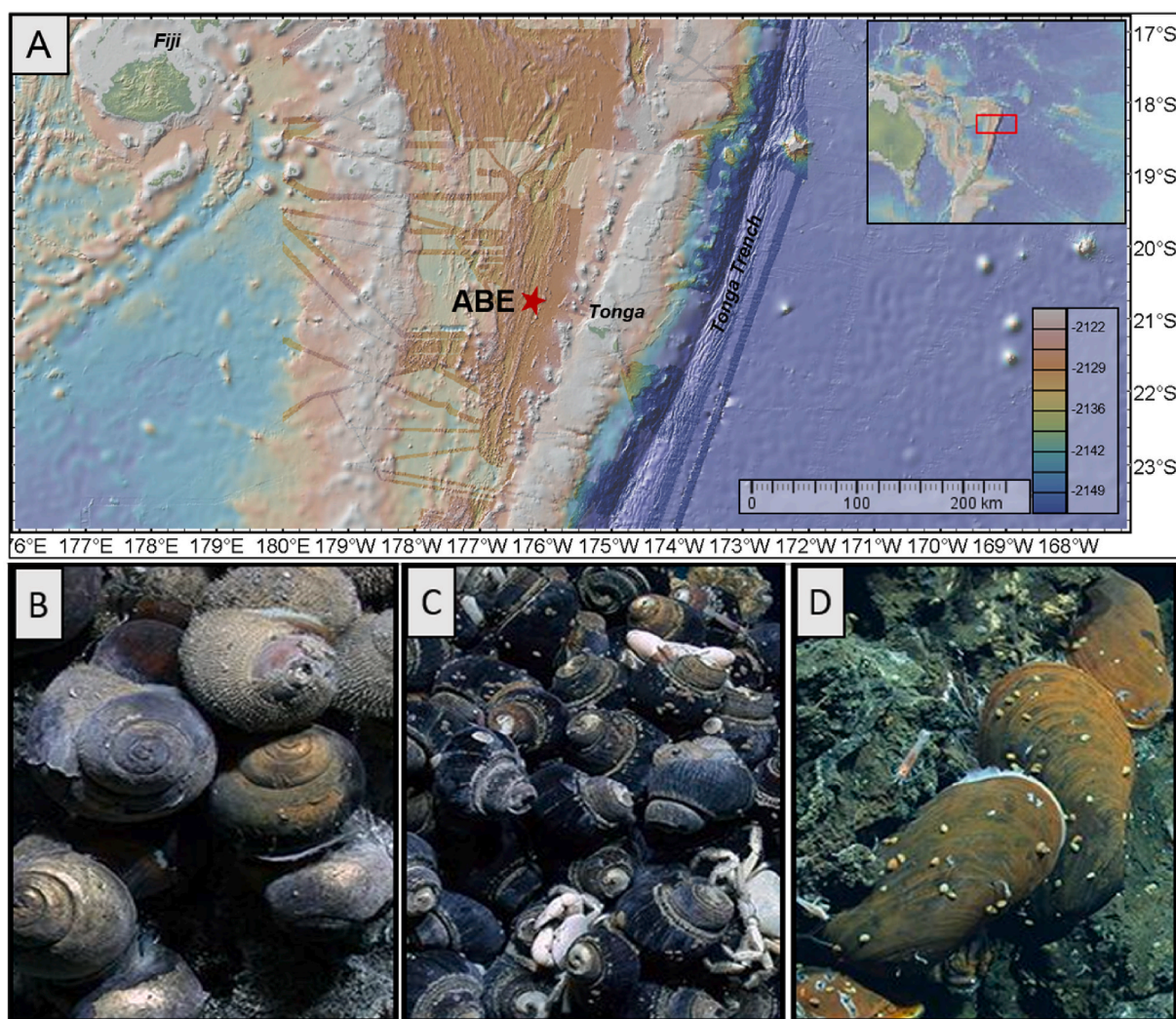


Fig. 1. Map of study location, the ABE hydrothermal vent field denoted by a red star, situated between Fiji and Tonga. B-D) Dominant megafauna associated with diffuse flow: B) *Alviniconcha hessleri*, C) *Ifremeria nautiliei*, and D) *Bathymodiolus septemdiarium*. Map created with GeoMapApp ([www.geomapapp.org/](http://www.geomapapp.org/))/CC BY. Images B-D from Du Preez and Fisher (2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



known to provide approximately 50% to vent biodiversity (Zekely et al., 2006a, c; Gollner et al., 2015) but they are also thought to play an important trophic role in vent food webs (Limén et al., 2007), e.g. *Stygiopontius quadrispinosus*, which was present in samples of the paralvinellid worm *Paralvinella sulfincola*, was found in abundances ranging from 4 to 1853 copepod individuals per worm (Limén et al., 2008).

A total of 34 studies have investigated the diversity of meiofauna (animals between 32  $\mu\text{m}$  and 1 mm in size) at vents. Nine of these were conducted on the Mid-Atlantic ridge (Zekely et al., 2006a, c; Cuvelier et al., 2014; Sarrazin et al., 2015; Zeppilli et al., 2015; Plum et al., 2017; Baldrighi et al., 2018; Alfaro-Lucas et al., 2020; Spedicato et al., 2020). Thirteen have been conducted along the East Pacific Rise (Dinet et al., 1988; Tsurimi et al., 2003; Flint et al., 2006; Zekely et al., 2006a, c; Copley et al., 2007; Degen et al., 2012; Gollner et al., 2006, 2007, 2010b, 2013, 2020) and five along the Juan de Fuca Ridge in the Northeastern Pacific Ocean (Tsurimi, 2001, 2003; Limén et al., 2007, 2008; Lelièvre et al., 2018). More recently, focusing on copepod diversity, sex ratios and new descriptions of dirivultids, seven have been conducted on the Northwest Pacific Ocean (Uejima et al., 2017; Senokuchi et al., 2018, 2020; Uyeno et al., 2018; Nomaki et al., 2019; Ivanenko et al., 2018, 2019), and three new descriptions of dirivultids and tegastids have come from the Indian Ridge (Ivanenko et al., 2019; Lee et al., 2020; Kim and Lee, 2020). Only two studies have focused on West Pacific back-arc basins (Shirayama, 1992; Vanreusel et al., 1997), highlighting that back-arc basins are clearly understudied (Levin et al., 2016) with regards to meiofauna, especially copepods.

Copepods are the second most abundant meiofauna taxon in most aquatic systems and can be the most abundant taxonomic group in deep-sea diffuse vent ecosystems where hard substrate is available (Gollner et al., 2010a). Most of the Copepoda at vents belong to the order Siphonostomatoida which currently contains 60 families. Of these the diverse family Dirivultidae which currently has 78 described species in the World Register of Marine Species (WORMS), including 9 recent additions from Uyeno et al. (2018) and Lee et al. (2020) ([marinespecies.org](http://marinespecies.org); accessed on December 8, 2020), contains most of the vent-endemic, globally distributed copepods which have successfully adapted to extreme vent conditions (Gollner et al., 2010a), although (albeit at lower abundances) specimens have been collected from vent-peripheries where conditions are near ambient (Gollner et al., 2020).

Despite their importance we lack knowledge on the abundance and species richness of copepods in the entire Lau-Tonga back-arc region, although hydrothermal vents in this area are a potential target for deep-sea mineral exploitation. In fact, the entire ABE field is situated within the area contracted for deep-sea mineral mining exploration, previously granted to Nautilus Minerals Inc, later acquired by Deep-Sea Mining Finance (DSMF) (<https://dsmf.im/>; accessed on June 24, 2020). DSMF has to date secured exploration rights to 25 Seafloor Massive Sulfide (SMS) systems in the Kingdom of Tonga (<https://dsmf.im/>; accessed on June 24, 2020). The decadal stability of megafauna distribution within the ABE vent field (Du Preez and Fisher, 2018), raises questions on the resilience of meiofauna communities and the ecosystem's ability to recover after disturbance.

The baseline knowledge of biodiversity, and community dynamics should be increased for this region in order to better quantify the importance of meiofauna in stable chemosynthetic systems and to properly inform policy making for deep-sea mining. This is the first study to quantitatively assess the diversity of copepod communities associated with megafauna at the ABE vent within the Lau Basin. We investigate how these communities are structured along a physicochemical stress gradient of hydrothermal vent diffuse flow in the three main foundation species associated with this vent system (i.e., *Alviniconcha hessleri*, *Ifremeria nautilei*, and *Bathymodiolus septemdiarium*).

## 2. Methods

### 2.1. Study site

The study area is situated within the Lau Basin, a back-arc basin located in the Southwest Pacific between the Lau ridge and the Tonga ridge. ABE (20° 45'S and 176° 11'E) (Fig. 1A) is characterized by a spreading rate of 39–73 mm year<sup>-1</sup> and an andesitic geological setting (Martinez et al., 2006). It consists of 6 hydrothermal vent sites, two of which are diffuse. Samples were taken from the specific ABE1 diffuse venting site at a depth of 2142 m (Ferrini et al., 2008; Fig. 7; Podowski et al., 2010; Sen et al., 2014).

### 2.2. Sampling

#### 2.2.1. Collection methods

All samples were collected in May–June 2009 using the remotely operated vehicle (ROV) *Jason II* from aboard the research vessel (R/V) *Thomas G. Thompson* (during dive numbers 425, 426, and 427). Diffuse flow sites within the ABE field are characterized by three distinct foundation species, *Alviniconcha hessleri*, *Ifremeria nautilei* and *Bathymodiolus septemdiarium* (Fig. 1B–D). Three faunal samples were taken in each habitat using a quantitative mussel pot sampling device with a surface area of 531 cm<sup>2</sup> (Van Dover, 2002).

An in-situ voltammetry system with a wand that housed a thermocouple and four Au/Hg working electrodes was used for measuring temperature, oxygen concentration and sulfide concentration (Luther et al., 2001, 2008). The voltammetry system was both positioned by the ROV arm directly onto the snail and mussel aggregations before the faunal collection with the mussel pot was carried out, and positioned by the ROV arm into the faunal hole that was created by the mussel pot directly after faunal collection. With this method, the range of abiotic conditions that is experienced by the mobile associated fauna living on, underneath and amongst the megafauna, can be characterized. This collection further provides insight into habitat amelioration by megafauna.

At each discrete location, 5–7 voltammetric scans were taken, providing a range of temperature and concentration measurements at each location from which the minimum, maximum, and average temperatures, oxygen and sulfide concentrations could be calculated. The minimum detectable and quantifiable concentration of sulfide was 0.2  $\mu\text{mol L}^{-1}$ . Undetectable sulfide was treated as half the minimum detectable concentration (0.1  $\mu\text{mol L}^{-1}$ ) as per Podowski et al. (2009, 2010) and Sen et al. (2013, 2014). The minimum detectable concentration of oxygen was 5  $\mu\text{mol L}^{-1}$ , and the minimum quantifiable concentration of oxygen was 15  $\mu\text{mol L}^{-1}$ . Following Podowski et al. (2009, 2010) and Sen et al. (2013, 2014) half the minimum detectable concentration (2.5  $\mu\text{mol L}^{-1}$ ) was used for points with undetectable oxygen, and points with detectable but not quantifiable oxygen concentrations were treated as the average of detectable and quantifiable limits (10  $\mu\text{mol L}^{-1}$ ) for statistical analyses.

#### 2.2.2. Sample processing

On board, faunal samples were processed by washing the megafauna samples with 32  $\mu\text{m}$  filtered seawater onto a 1 mm and 32  $\mu\text{m}$  sieve. Animals retained on the 32  $\mu\text{m}$  sieve were fixed in 4–8% buffered Formaldehyde and transferred to 70% EtOH for storage. The sorting and counting of copepods were conducted using a stereomicroscope, and these were then transferred to glycerine and mounted on slides for taxonomic identification. Copepods were identified under a light microscope using a variety of literature (Lang, 1948; Humes, 1980, 1987, 1999; Higgins and Thiel, 1988; Huys et al., 1996; Heptner and Ivanenko, 2002; Desbruyères et al., 2006; Giere, 2009). For representative

sub-sampling, 200 individuals were identified from each sample until an asymptote was reached in cumulative species effort curves. Copepodites were counted and included in this study as “copepodites”, but not identified to species level. The sex of the most abundant copepod species, *Stygiopontius lauensis*, was identified and relative abundances analysed. The sexes of the other two dirivultid species were also determined during identification, but only the sex ratios of the most abundant organism are reported in this study.

### 2.2.3. Volume and biomass

The size of each individual copepod was measured using ImageJ (version 1.52a) from high resolution (300 dpi) photographs taken with the Leica camera installed on a light microscope. Up to 30 randomly selected individuals were measured from each species to obtain a nominal size (species average). The full length (from the end of the rostrum to the last urosomal segment) and maximum width of each individual were measured. The volume of siphonostomatoid and harpacticoid copepods was calculated as  $\text{Volume} = \text{Length} \times \text{Width}^2 \times \text{Conversion factor}$  corresponding to body form as described in Warwick and Gee (1984). For harpacticoids the body form was identified mostly as either semi-cylindrical compressed, semi-cylindrical or fusiform. For the siphonostomatoids this body form was defined as pyriform depressed (see Warwick and Gee, 1984, for numerical details). Volumes were multiplied by the specific gravity of 1.13 (for meiobenthic organisms in general) to obtain wet mass in mg (see Feller and Warwick, 1979). For siphonostomatoids, this was compared to the methods derived from the Chislenko nomograms (Chislenko, 1968) and the biomass fell within the range for one individual (0.04–0.09 mg). The total biomass of copepods per 531 cm<sup>2</sup> and 10 cm<sup>2</sup> was calculated by multiplying the mean biomass of each species by the number of specimens in that sample once abundances were established. The biomass of megafauna (wet mass in g) was also calculated in order to determine the biomass of copepods per that of megafauna.

## 2.3. Data analysis

### 2.3.1. Diversity and community composition

After copepods were counted, abundance was standardized to a 10 cm<sup>2</sup> area in order to be used in comparison with other studies. Species richness (S), calculated as the number of species within a given sample, Shannon diversity index ( $H'_{\log_e}$ ), and Pielou's evenness index ( $J'$ ) were calculated using the ‘vegan’ package in R, version 3.5.2 (Oksanen et al., 2018). Size-based rarefaction and extrapolation of species richness after identifying 3000 ind. (Sm3000) and of species richness at a sample coverage of 98% (SCm0.98) was obtained via the iNEXT package in R version 3.5.2. (Chao and Jost, 2012).

Differences in abundance 10 cm<sup>-2</sup> (square root transformed), S (square root transformed),  $H'_{\log_e}$  (no transformation),  $J'$  (no transformation), and abiotic variables were investigated using several methods to account for the small sample numbers ( $n = 3$ ) per habitat. Normality of the diversity indices was tested using the Shapiro test function in R version 3.5.2, resulting in  $p$  values  $> 0.05$ , indicating that the distribution of the data is not significantly different from the normal distribution. A bootstrapped (10,000 resamplings) Welch's independent 2-group  $t$ -test was then used to test the differences of the diversity indices between the habitats, as it is robust to small sample numbers associated with large, unequal variance (Derrick and White, 2016), both of which apply to the data. In addition, the non-parametric Kruskal Wallis test was used with post hoc multiple comparisons (Dunn's pairwise test with Bonferroni adjustment). To evaluate similarities and dissimilarities of samples Bray-Curtis similarity was calculated from the standardized abundance data which were square root transformed to down-weight the importance of very abundant species without losing the influence of rarer species (Clarke and Warwick, 2001). Similarity percentage (SIMPER) analyses and analysis of similarity (ANOSIM) were conducted. Non-metric multi-dimensional scaling (nMDS) was

performed to visualise the level of similarity of individual species in the community dataset using the ‘vegan’ packages in R version 3.5.2.

### 2.3.2. Effects of environmental variables on community composition and diversity

The BIOENV function in R (Clarke and Ainsworth, 1993) was used to identify which environmental variables best correlate to sample similarities of the biological community (abundance 10 cm<sup>-2</sup>). The similarity matrix of the community was fixed, while subsets of the environmental variables were used to calculate the environmental similarity matrix. A correlation coefficient (typically Spearman rank correlation coefficient in the BIOENV function) was then calculated between the two matrices and the best subset of environmental variables was selected. Pearson's rank correlation analysis was then used to investigate the strength of the relationship of the environmental variables and the diversity indices, based on Pearson's correlation coefficient ( $r$ ). A Pearson's rank correlation analysis was, beforehand, used to investigate the relationship between the environmental variables. Due to covariance of temperature and H<sub>2</sub>S/HS<sup>-</sup> in this study ( $r = 0.95$ ,  $p < 0.0001$ ), only H<sub>2</sub>S/HS<sup>-</sup> and oxygen were retained for further analysis.

## 3. Results

### 3.1. Physio-chemical measurements

Temperatures were highest at the *Alviniconcha* habitat (mean 21–28 °C), lower at the *Ifremeria* habitat (mean 7–18 °C), and lowest at the *Bathymodiolus* habitat (mean 4–9 °C) (Table 1). Temperatures did not differ significantly among the habitats but were always higher in the faunal hole with recorded means of 41–57 °C inside the *Alviniconcha*, 21 °C inside the *Ifremeria* and 9–11 °C inside the *Bathymodiolus* (Table 2).

Sulfide concentrations were highest at the *Alviniconcha* habitat (mean 107–151 μmol L<sup>-1</sup>), lower at the *Ifremeria* habitat (mean 4–33 μmol L<sup>-1</sup>), and lowest at the *Bathymodiolus* habitat (mean 2–9 μmol L<sup>-1</sup>) (Table 1), differing significantly from concentrations at the *Alviniconcha* habitat ( $p = 0.02$ ). Sulfide concentrations were always higher in the faunal hole with recorded mean concentrations of 240–343 μmol L<sup>-1</sup> inside the *Alviniconcha* (over twice the amount as outside of the faunal hole), 43 μmol L<sup>-1</sup> inside the *Ifremeria* and 15–21 μmol L<sup>-1</sup> inside the *Bathymodiolus* (Table 2).

Oxygen concentrations were lowest at the *Alviniconcha* habitat (mean 3–11 μmol L<sup>-1</sup>), slightly higher at the *Ifremeria* habitat (mean 22–103 μmol L<sup>-1</sup>), and highest at the *Bathymodiolus* habitat (mean 71–134 μmol L<sup>-1</sup>) (Table 1). Values at the *Bathymodiolus* habitat were significantly higher than at the *Alviniconcha* habitat ( $p = 0.03$ ). Oxygen concentrations were always lower in the faunal hole (Table 2); inside of the *Alviniconcha* and *Ifremeria* holes, only the minimum detectable concentration was recorded (2.5 μmol L<sup>-1</sup>), however inside of the *Bathymodiolus* hole, mean oxygen concentrations reached (62–105 μmol L<sup>-1</sup>).

### 3.2. Diversity and community composition

A total of 3981 individual copepods from a total sampling area of 4779 cm<sup>2</sup> in 9 samples were counted. The highest abundance of copepods was encountered at the *Alviniconcha* habitats ranging from 11 to 30 ind. 10 cm<sup>-2</sup> (mean ± standard deviation: 17 ± 4 ind. 10 cm<sup>-2</sup>). Intermediate abundances were encountered at the *Ifremeria* habitats ranging from 1 to 7 ind. 10 cm<sup>-2</sup> (mean ± standard deviation: 4 ± 1 ind. cm<sup>-2</sup>). The lowest abundance was encountered at the *Bathymodiolus* habitats ranging from 2 to 4 ind. 10 cm<sup>-2</sup> (mean ± standard deviation: 4 ± 0.4 ind. cm<sup>-2</sup>) (Table 3). No significant differences in abundance were found between any of the three habitats (Table 4). From the total of 9 samples, 18 species were identified. The most abundant copepods were those of the order Siphonostomatoida, with 89% of the individuals

**Table 1**

Physiochemical characteristics at the megafaunal assemblages, showing location (longitude and latitude), number of scans (N) taken at each location, the mean ( $\pm 95\%$  confidence intervals), minimum, and maximum temperature, oxygen and hydrogen sulfide measurements taken at each of the sample locations. The sample column refers to the three collections taken within each of the *Alviniconcha* (Alv1-3), *Ifremeria* (If1-3), and *Bathymodiolus* (Mu1-3) habitats.

Sample	Location		Number of reads/scans N	Temperature ( $^{\circ}\text{C}$ )			Oxygen ( $\mu\text{mol}\cdot\text{L}^{-1}$ )			$\text{H}_2\text{S}/\text{HS}^-$ ( $\mu\text{mol}\cdot\text{L}^{-1}$ )		
	Latitude	Longitude		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Alv1	$-20^{\circ} 45' 47.7''$	$-176^{\circ} 11' 29''$	22	21.1 ( $\pm 5.8$ )	8	41.5	9.7 ( $\pm 7.8$ )	2.5	93	107.5 ( $\pm 38.9$ )	14.9	265.6
Alv2	$-20^{\circ} 45' 47.7''$	$-176^{\circ} 11' 28.9''$	22	27.9 ( $\pm 3.3$ )	17	46.0	2.5 ( $\pm \text{N/A}$ )	2.5	62.5	151.1 ( $\pm 21.5$ )	56.4	264.4
Alv3	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.9''$	34	22.3 ( $\pm 3.4$ )	8.5	42.5	11.1 ( $\pm 8.1$ )	2.5	0	107.2 ( $\pm 26.2$ )	26	227.7
If1	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.6''$	30	18 ( $\pm 1.1$ )	7	26	22.6 ( $\pm 9.8$ )	2.5	91.1	32.7 ( $\pm 13.9$ )	5.3	226.8
If2	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.6''$	24	6.9 ( $\pm 1.8$ )	3.5	19	103.5 ( $\pm 21.6$ )	2.5	162.8	8.9 ( $\pm 3$ )	3	23.8
If3	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.7''$	21	11.5 ( $\pm 1.1$ )	4	11.5	80.4 ( $\pm 17.1$ )	2.5	147.8	3.7 ( $\pm 3$ )	2.1	24.1
Mu1	$-20^{\circ} 45' 47.6''$	$-176^{\circ} 11' 28.9''$	28	8.8 ( $\pm 1.1$ )	3.5	13	71.4 ( $\pm 19.2$ )	2.5	168.4	8.6 ( $\pm 2.5$ )	0.5	20
Mu2	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.9''$	58	3.7 ( $\pm 0.1$ )	3	4.5	134.4 ( $\pm 3.8$ )	100.3	165.6	2.4 ( $\pm 0.4$ )	0	4.7
Mu3	$-20^{\circ} 45' 47.5''$	$-176^{\circ} 11' 28.8''$	51	5.7 ( $\pm 0.9$ )	2.5	15	125.8 ( $\pm 13$ )	27.3	183.3	4.60 ( $\pm 1.3$ )	0.4	17

**Table 2**

Physiochemical characteristics inside the megafaunal assemblages, after megafauna has been removed with the faunal collection device (inside the faunal hole), showing location (longitude and latitude), number of scans (N) taken at each location, the mean ( $\pm 95\%$  confidence intervals), minimum and maximum temperature, oxygen and hydrogen sulfide measurements. The sample column refers to the three collections taken within each of the *Alviniconcha* (Alv1-3), *Ifremeria* (If1-3), and *Bathymodiolus* (Mu1-3) habitats.

Sample	Location		Number of reads/scans N	Temperature ( $^{\circ}\text{C}$ )			Oxygen ( $\mu\text{mol}\cdot\text{L}^{-1}$ )			$\text{H}_2\text{S}/\text{HS}^-$ ( $\mu\text{mol}\cdot\text{L}^{-1}$ )		
	Latitude	Longitude		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Alv1	$-20^{\circ} 45' 47.7''$	$-176^{\circ} 11' 29''$	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Alv2	$-20^{\circ} 45' 47.7''$	$-176^{\circ} 11' 28.9''$	13	57.3 ( $\pm 5.4$ )	27	65.5	2.5	2.5	2.5	343.2 ( $\pm 31.5$ )	283.7	491
Alv3	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.9''$	7	41.3 ( $\pm 5.6$ )	27	48	2.5	2.5	2.5	239.5 ( $\pm 24.1$ )	181.8	278.2
If1	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.6''$	16	20.6 ( $\pm 2.6$ )	11.5	25	2.5	2.5	2.5	43.3 ( $\pm 6.2$ )	9.4	66.2
If2	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.6''$	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
If3	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.7''$	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mu1	$-20^{\circ} 45' 47.6''$	$-176^{\circ} 11' 28.9''$	22	8.4 ( $\pm 2.9$ )	3	21.5	104.9 ( $\pm 30.5$ )	2.5	171.1	14.8 ( $\pm 7.6$ )	0.4	45.3
Mu2	$-20^{\circ} 45' 47.8''$	$-176^{\circ} 11' 28.9''$	28	7.8 ( $\pm 1.7$ )	3	18.5	86.6 ( $\pm 22.4$ )	2.5	173.3	17 ( $\pm 8.6$ )	1	81.4
Mu3	$-20^{\circ} 45' 47.5''$	$-176^{\circ} 11' 28.8''$	32	10.6 ( $\pm 2.4$ )	3	22.5	62 ( $\pm 23.9$ )	2.5	186.6	20.9 ( $\pm 5.5$ )	1.4	50.4

identified belonging to this order, followed by harpactoid copepods, represented by 8% of the individuals in the community. Copepodites made up 2% of the community and nauplii made up 0.3%. The orders Calanoida and Cyclopoida made up 0.2% and 0.1% of the community, respectively.

The species *Stygiopontius lauensis*, belonging to the Dirivultidae family, dominated the *Alviniconcha* habitat with a relative abundance of  $>90\%$  across all three *Alviniconcha* samples. *S. lauensis* was also found to be the dominant species at the *Ifremeria* habitats albeit at lower abundances than in the *Alviniconcha* habitats, with a relative abundance of 26–88%. At the *Bathymodiolus* habitat, the dominant species was also found to be *S. lauensis*, with relative abundances of 1–65% closely followed by *Amphiascus* aff. *varians* with relative abundances of 3–38%. *Ameira* sp. 1, was found in relative abundances of 15–27% and members of the Tisbidae family were found in relative abundances of 4–16% (Table 3).

Similar trends were encountered for the three diversity indices calculated across the three habitats,  $S$ ,  $H'_{(\log_e)}$  and  $J'$  increasing with decreasing influence of hydrothermal fluid emissions (Table 3). Negligible variations in abundance were observed.  $S$ ,  $H'_{(\log_e)}$ , and  $J'$  were significantly higher in the *Bathymodiolus* habitats than in the *Alviniconcha* habitats ( $p < 0.05$ ) and  $H'_{(\log_e)}$ , and  $J'$  were significantly higher in the *Bathymodiolus* habitats than the *Ifremeria* habitats. Abundance was similar between the habitats (Table 4; bootstrapped Welch's  $t$ -test). The results of the Kruskal Wallis test with post-hoc multiple comparisons yielded no significant differences for any of the diversity indices between the habitats, however alpha approximated 0.05 for  $S$  between *Ifremeria* and *Bathymodiolus* habitats and for  $H'_{(\log_e)}$ , and  $J'$  between *Alviniconcha* and *Bathymodiolus* habitats (Table A.1). The iNEXT results showed that sampling effort was sufficient, as both  $S$  and  $H'_{(\log_e)}$  reached

an asymptote at the *Alviniconcha* and *Ifremeria* habitats. The same was true for  $H'_{(\log_e)}$  at the *Bathymodiolus*. However,  $S$  at the *Bathymodiolus* did not reach an asymptote (Fig. 2). A sample coverage of 0.98 ( $S_{\text{Cm}0.98}$ ) was obtained for every sample except for the most diverse sample Mu1 (*Bathymodiolus*), indicating that although overall sample number was low (3 samples per habitat), the number of individuals collected and identified per sample was sufficient (with the exception of Mu1) for accurate comparison between the habitats (Fig. B).

The outcome of the SIMPER analysis revealed that copepod communities at the *Alviniconcha* and *Ifremeria* habitat were 60% dissimilar, the *Ifremeria* and *Bathymodiolus* habitats were 75% dissimilar, and *Alviniconcha* and *Bathymodiolus* habitats were 89% dissimilar (Table 4). In each case, *S. lauensis* was the most prevalent species contributing the most to the average dissimilarity between the habitats (*Alviniconcha*-*Ifremeria* = 55%, *Ifremeria*-*Bathymodiolus* = 39% *Alviniconcha*-*Bathymodiolus* = 73%). The results of the ANOSIM analysis demonstrated that there were significant differences in the community structure between all three habitats (ANOSIM:  $R = 0.6$ ,  $p = 0.005$ ). The results of the non-metric Multidimensional Scaling (nMDS) ordination plot with overlaid environmental factors as scaled vectors, revealed that copepods from the distinct megafauna habitats also clustered based on the directionality of the environmental vectors (Fig. 3).

### 3.3. Effects of environmental variables on diversity and community composition

The results of the BIOENV analysis indicate that the greatest dissimilarity of the community composition was explained best by variation in oxygen concentrations (Spearman's  $p = 0.65$ ), followed by a combination of oxygen and temperature (Spearman's  $p = 0.58$ ) and

**Table 3**

Biotic characteristics including abundance, biomass, and the diversity indices species richness (S), Shannon diversity ( $H'_{\log e}$ ), Pielou's evenness index ( $J'$ ),  $\hat{C}_{Sobs}$  (mean percent sample coverage),  $S_{(m3000)}$  (species richness at a sample size of 3000 ind.), and  $S_{Cm0.98}$  (species richness at a sample coverage of 98%). In addition, relative abundance of higher copepod taxa and copepod species is given. The three samples at the *Alviniconcha* communities are denoted by the sub-headers Alv1-Alv3, for the *Ifremeria* communities by If1-If3, and for the *Bathymodiolus* communities by Mu1-Mu3.

Habitat Samples	<i>Alviniconcha</i> communities			<i>Ifremeria</i> communities			<i>Bathymodiolus</i> communities		
	Alv1	Alv2	Alv3	If1	If2	If3	Mu1	Mu2	Mu3
<b>Abundance (no. individuals)</b>									
Total ab. per sample area (531 cm <sup>-2</sup> )	610	1613	562	392	44	244	201	199	116
Ab. 10 cm <sup>-2</sup>	11	30	11	7	1	5	4	4	2
<b>Biomass [mg wet weight]</b>									
Copepod biomass [mg wet weight 531 cm <sup>-2</sup> ]	36.4	96.3	33.2	23.3	2.3	13.2	4.8	2.7	4.9
Mega fauna biomass [g wet weight 531 cm <sup>-2</sup> ]	1480.8	802.8	494.1	1672.8	3075.8	3184.5	572.1	2523.7	2278.4
Copepod biomass [mg wet weight 10 cm <sup>-2</sup> ]	0.7	1.8	0.7	0.4	0.04	0.2	0.1	0.1	0.1
<b>Relative abundance of higher copepod taxa (%)</b>									
Siphonostomatoida	99.5	99.5	98	99	86	90	26.5	2.5	65
Harpacticoida	0	0	0	0	2.5	1	68	95	29
Calanoida	0	0	0	0	0	0	0.5	0	1
Cyclopoida	0	0	1	0	0	0	2	0	0
Copepodite	0.5	0.5	1	1	11.5	9	3	2.5	5
<b>Relative abundance of copepod species (%) Siphonostomatoida-Dirivultidae</b>									
<i>Stygiopontius lauensis</i>	99	93	93	99	86	88	26	1	65
<i>Stygiopontius brevispina</i>	<0.01	1	2	0	0	2	0	0	0
<i>Chasmatopontius</i> sp.1	0	5	4	0	0	0	<0.01	2	0
<i>Ameira</i> sp.1	0	0	0	0	2	1	18	27	15
<i>Ameira</i> sp. 2	0	0	0	0	0	0	1	4	0
<i>Stenocopia longicaudata</i>	0	0	0	0	0	0	6	2	0
<i>Ameiropsis</i> sp.1	0	0	0	0	0	0	1	1	4
<b>Harpacticoida-Laophontidae</b>									
<i>Bathylaophonte</i> sp.1	0	0	0	0	0	0	1	1	0
<b>Harpacticoida-Miraciidae-Diosaccinae</b>									
<i>Amphiascus</i> aff. <i>varians</i>	0	0	0	0	0	0	23	38	3
<i>Macrosetella</i> sp.1	0	0	0	0	0	0	<0.01	0	0
<b>Harpacticoida-Tisbidae</b>									
Tisbidae sp.1	0	0	0	0	0	0	8	16	4
<b>Harpacticoida-Ectinosomatidae</b>									
<i>Microsetella norvegica</i>	0	0	0	0	0	0	<0.01	0	0
<i>Halectinosoma</i> sp.1	0	0	0	0	0	0	3	1	0
<i>Halectinosoma</i> sp. 2	0	0	0	0	0	0	3	5	3
<i>Halophytophilus</i> sp.1	0	0	0	0	0	0	<0.01	0	0
Copepodite	<0.01	<0.01	1	1	11	9	3	3	5
<b>Cyclopoida-Oithonida</b>									
Oithonidae sp.1	0	0	0	0	0	0	0	0	1
<i>Cyclopina elegans</i>	0	0	0	0	0	0	<0.01	0	0
<b>Calanoida</b>									
Calanoida sp.1	0	0	1	0	0	0	1	0	0
<b>Diversity Indices</b>									
$S_{obs}$	3.0	4.0	5.0	2.0	3.0	5.0	17.0	12.0	8.0
$S_{m(3000)}$	3 ± 0.3	4 ± 0.02	5 ± 0.3	2 ± 0.1	3 ± 0.5	5 ± 1.3	17 ± 10.2	12 ± 7.2	8 ± 0.6
$\hat{C}_{Sobs}$	100.0	100.0	100.0	100.0	100.0	99.9	97.5	98.5	100.0
$S_{Cm0.98}$	1.0	3.0	4.0	1.0	3.0	3.0	20.0	11.0	8.0
$H'_{(\log e)}$	0.1	0.3	0.4	0.1	0.5	0.5	2.1	1.8	1.2
$J'$	0.1	0.2	0.2	0.1	0.4	0.3	0.7	0.7	0.6

**Table 4**

p-values of pairwise bootstrapped (10,000 resamplings) Welch's *t*-test showing significant differences in diversity indices between the habitats for species richness (S), Shannon diversity ( $H'_{\log e}$ ), and Pielou's evenness index ( $J'$ ) with lower and upper 95% confidence intervals (LCI and UCI, respectively). In addition, SIMPER percentage dissimilarity between megafaunal assemblages *Alviniconcha* (Alv), *Ifremeria* (If) and *Bathymodiolus* (Mu) is given. Values in bold are significant.

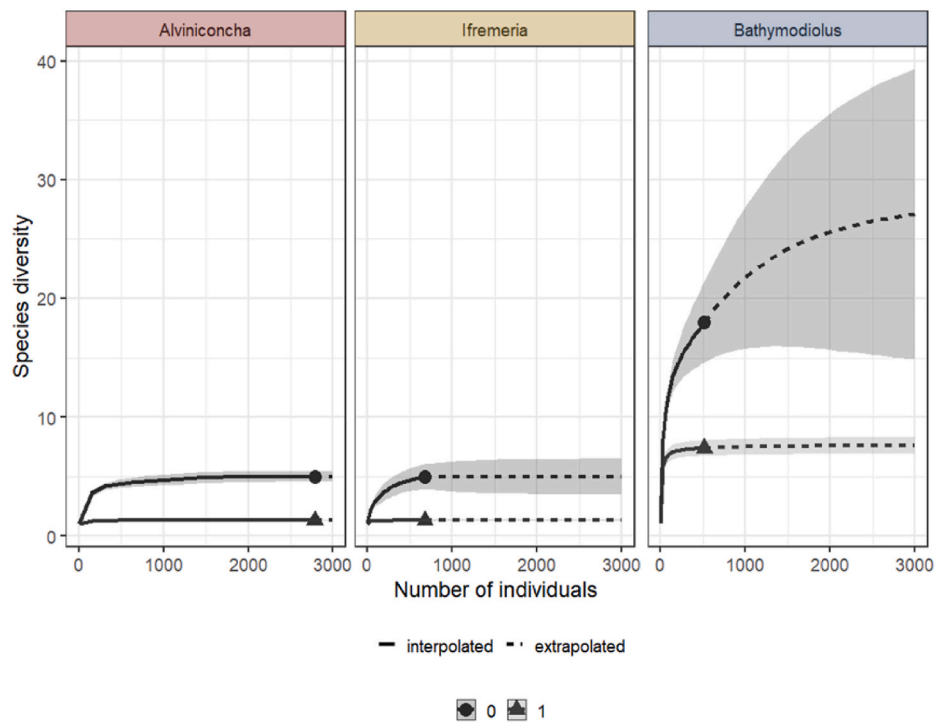
Sites	Ab.	LCI	UCI	S	LCI	UCI	$H'_{(\log e)}$	LCI	UCI	$J'$	LCI	UCI	Diss. (%)
Alv-If	0.1	0.8	3.4	0.5	-0.2	0.60	0.6	-0.3	0.2	n/a	n/a	n/a	60
If-Mu	0.9	-0.9	0.9	0.05	-2.1	-1.02	<b>0.03</b>	-1.6	-0.9	<b>0.04</b>	-0.5	-0.3	75
Alv-Mu	0.1	1.4	3.7	<b>0.04</b>	-1.9	-0.90	<b>0.03</b>	-1.7	-1.1	<b>0.01</b>	-0.5	-0.4	89

temperature, oxygen and  $H_2S/HS^-$  (Spearman's  $p = 0.49$ ). Pearson's rank correlation analysis revealed that oxygen was correlated to abundance,  $H'_{(\log e)}$ , and  $J'$ . Fluctuations in  $H_2S/HS^-$  were found to covary significantly with changes in abundance and evenness, however a negligible relationship was found between  $H_2S/HS^-$  and S and  $H_2S/HS^-$  and  $H'_{(\log e)}$  (Fig. 4).

### 3.4. Size and biomass

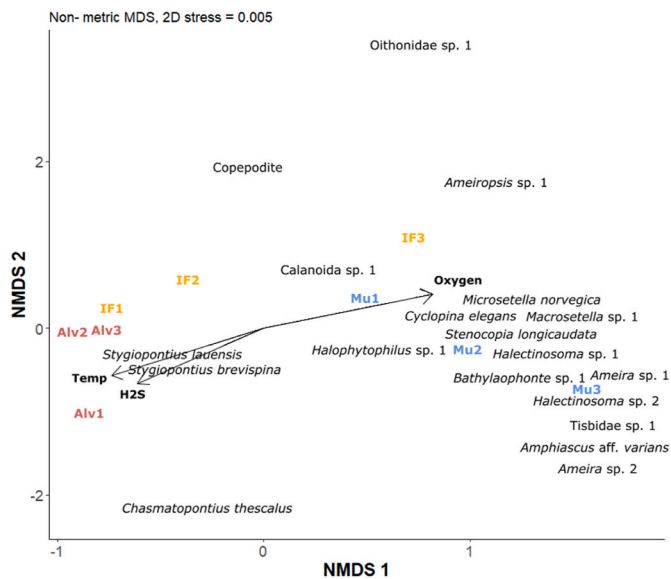
The largest species were *Ameira* sp. 2 and *Cyclopina elegans*. Of the Dirivultidae, *C. thescalus* had the biggest biovolume, followed by *S. lauensis* (Fig. 5). Within species, fluctuations in body size were negligible except for *S. lauensis* and *C. thescalus* whose female and male members differ in size as a result of sexual dimorphism, a trait found also in other species within the Dirivultidae (Tsurimi et al., 2003).

The total copepod biomass per total megafauna biomass was highest



**Fig. 2.** Interpolation (solid line) and extrapolation (dotted line) for  $S$  (0, triangle) and  $H'_{(\log_e)}$  (1, circle) as calculated with the iNEXT package in R (version 3.5.2), for a sample size of 3000 individuals. This was calculated for each habitat (*Alviniconcha*, *Ifremeria* and *Bathymodiolus*).





**Fig. 3.** Non-metric Multidimensional Scaling (nMDS) of community composition data (abundance  $10\text{ cm}^{-2}$ , square root transformed) across the *Alviniconcha* (Alv1-3), *Ifremeria* (If1-3), and *Bathymodiolus* (Mu1-3) sampling sites. Scaled vectors of environmental variables are fitted to the ordination using the envfit function in the ‘vegan’ package in R. The arrow shows the direction of the (increasing) gradient, and the length of the arrow is proportional to the correlation between the variable and the ordination.

at the *Alviniconcha* habitats, ranging from 33 to 96 mg of copepods per 494–1480 g of *Alviniconcha* snails. At the *Ifremeria* habitats, copepod biomass decreased, despite an increase in megafauna biomass, ranging from 2 to 23 mg of copepods per 1673–3185 g of *Ifremeria* snails, and the lowest biomass was found among the mussel beds, ranging from 3 to 5 mg of copepods per 572–2524 g of *Bathymodiolus* mussels. Per  $10\text{ cm}^2$ , copepod biomass ranged from 1 to 2 mg at the *Alviniconcha* habitat, 0.04–0.4 mg at the *Ifremeria* habitat and 0.05–0.09 mg at the *Bathymodiolus* habitat (Table 3).

### 3.5. Gender niche partitioning

The ratio of females to males (F:M) of *S. lauensis* was very high in the *Alviniconcha* habitats (26:1) decreasing drastically in the *Ifremeria* habitats (1:3) and increasing marginally in the *Bathymodiolus* habitats (1:2) (Fig. 6). Major differences in the number of females to males were found in the Alv2 sample (46:1), the If1 sample (1:14), and in the Mu2 sample which contained 100% females, however this increase in females was driven by the presence of only one female individual in the sample. No male members of *S. brevispina* were found in the samples and male members of *C. thescalus* were found only in samples Alv2 and Alv3, occurring in ratios of (1:1 and 2:1, respectively).

## 4. Discussion

Copepod communities are unique and clearly structured along the gradients of hydrothermal diffuse flow at the ABE vent site. Areas of high temperatures and high concentrations of  $\text{H}_2\text{S}/\text{HS}^-$  are dominated by the vent endemic species *S. lauensis* in very high abundance and include, albeit in considerably lower abundance, *S. brevispina*, and *C. thescalus*. With increasing oxygen concentrations,  $H'_{(\log_e)}$ ,  $J'$ , and  $S$  of copepods increased significantly, as conditions may become more tolerable to a wider range of species. Fluctuations in biomass were driven by the very high abundance of *S. lauensis*. Biomass decreased along the decreasing gradient, as did the ratio of females to males of this species, with more and larger females in the more extreme environmental conditions.

### 4.1. Abundance

Copepod abundance in this study was generally low ( $1\text{--}30\text{ ind. }10\text{ cm}^{-2}$ ), similar to that reported by Gollner et al. (2006) for the East Pacific Rise (EPR) ( $<1\text{--}31\text{ ind. }10\text{ cm}^{-2}$ ) and comparable to Zekely et al. (2006c) for the EPR ( $25\text{--}32\text{ ind. }10\text{ cm}^{-2}$ ) and the Mid-Atlantic Ridge (MAR) ( $\sim 15\text{ ind. }10\text{ cm}^{-2}$ ). The most abundant higher copepod taxon was the family Dirivultidae (order Siphonostomatoida). The relative abundance of dirivultid copepods in this study for areas of high flow ( $>90\%$  at *Alviniconcha* and *Ifremeria* habitats) exhibited low variability between the habitats and was driven by the species *S. lauensis*. The relative abundance of dirivultids is slightly higher but comparable to the Juan de Fuca Ridge (JFR) ( $\sim 80\%$ ) which was driven by the dirivultid species *S. quadrispinosus* (Tsurimi et al., 2003). Relative abundances of dirivultids were highly variable in the mussel habitats (26%, 1%, and 65% for the Mu1, Mu2, and Mu3 samples, respectively) but also comparable to those reported by Tsurimi et al. (2003) for low flow environments (60%) dominated by the dirivultid *Benthoxymus spiculifer*. These results are slightly higher than those reported by Zekely et al. (2006c) for the EPR ( $\sim 70\%$ , driven by *Aphotopontius mammilatus*) but comparable to those reported by Gollner et al. (2006) for the EPR with relative abundances of dirivultid copepods of  $>90\%$  at the mussel-dominated Buckfield site and 87–97% at the tubeworm dominated Tica site. Harpacticoid copepods made up 9.1% of the total copepod abundance in this study, within the range of that reported for tubeworm aggregations at the EPR (Zekely et al., 2006c: 3.8%; Gollner et al., 2006: 3–25%) but slightly higher than the mussel habitats of the EPR (Gollner et al., 2006: 3–4%). Mixed harpacticoid families identified by Tsurimi et al. (2003) in low temperature areas of the JFR are reported to have a collective relative abundance of 30%. These families include Ameiridae, Diosaccidae, Ectinosomidae, and Tisbidae, which were also found among the mussel habitats of the ABE site. The Diosaccidae (3–38%) and the Ameiridae (1–27%) contributed the most to the harpacticoid abundance in the mussel beds, similar to abundances in the JFR.

Copepod abundance in vent environments is on average  $< 80$  individuals per  $10\text{ cm}^2$  and copepods reportedly make up approximately 40% of total meiofauna communities associated with megafauna assemblages on hard substrates (Gollner et al., 2010b). The most abundant species in this study by far was *S. lauensis*, a copepod species belonging to a vent-endemic globally distributed genus that has also adapted to a wide range of temperatures and  $\text{H}_2\text{S}/\text{HS}^-$  concentrations. It was present in high abundances in all samples, with the highest being in areas of highest temperatures and  $\text{H}_2\text{S}/\text{HS}^-$  concentrations. Copepods in these abundances could provide a significant source of carbon in the food webs of hydrothermal vent systems (Limén et al., 2007; Limén, 2008). Limén (2008) highlights the potential importance of the Siphonostomatoida in the diet of paralvinellid worms, suggesting that highly abundant copepods may form a significant part of the diet of these organisms. It is therefore likely that they are a source of food for predatory organisms.

### 4.2. Diversity and community composition

A total of 18 copepod species were identified in this study. The number of copepod species identified here is slightly higher than reported by Zekely et al. (2006c) for the EPR (14) and the MAR (6) but lower than that reported by Gollner et al. (2006) for the EPR (27). At ABE,  $H'_{(\log_e)}$ ,  $J'$ , and  $S$  increased significantly with increasing oxygen concentrations. Habitat generalists were found among the *Bathymodiolus* habitat, with many genera also reported from shallow water coastal environments, shallow vent environments and peripheral vent environments as well as vent sediments (Gollner et al., 2017; Plum et al., 2017; Zeppilli et al., 2018). These species include *Ameira* sp. 1 and 2, and *Bathylaophonte* sp. 1. *Amphiascus* and genera belonging to the Tisbidae family, are more cosmopolitan, found across a wide range of marine



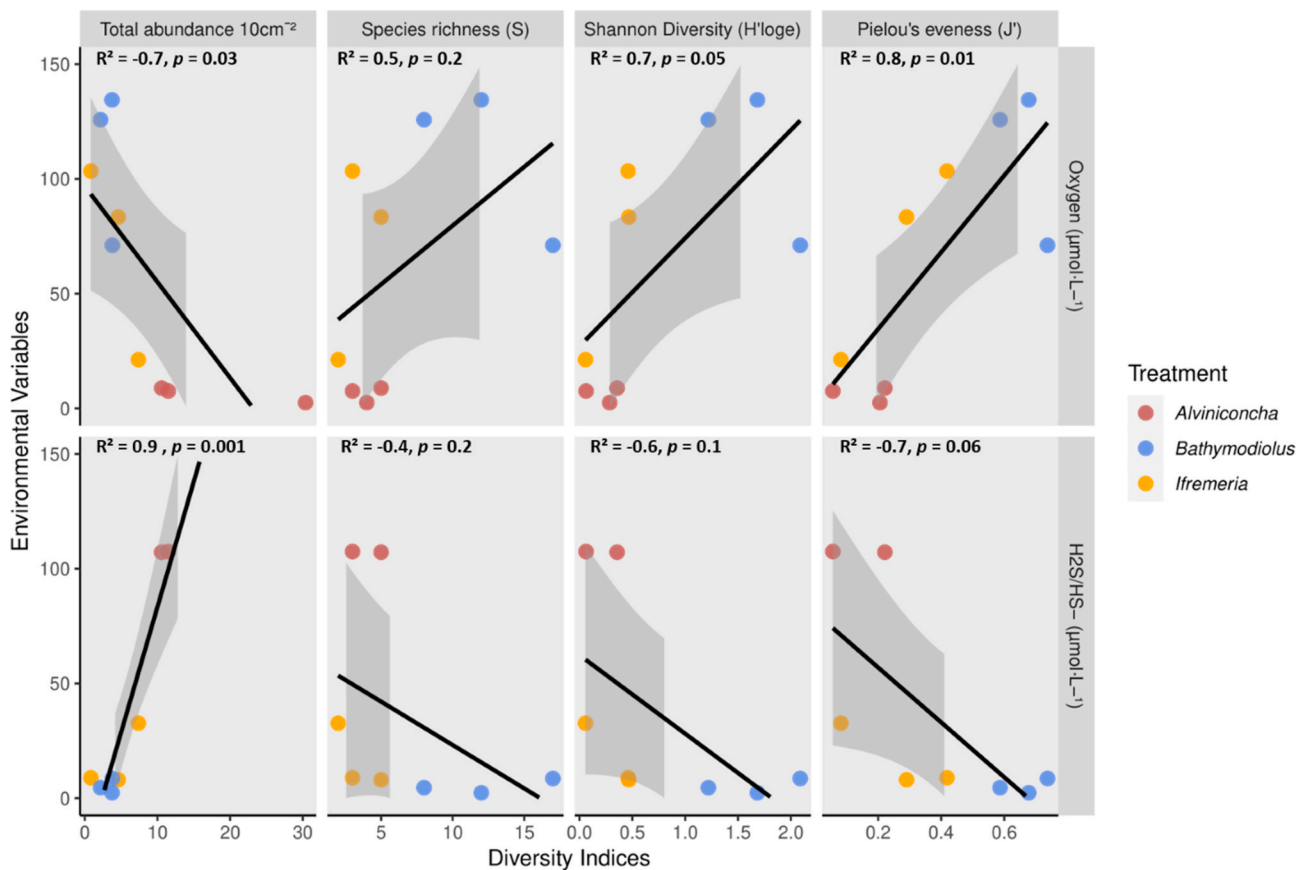


Fig. 4. Pearson's Rank correlation plots to visualise correlation of mean Oxygen and H<sub>2</sub>S/HS- concentrations among megafaunal aggregations with abundance and diversity indices. Grey scale = 95% confidence intervals.

environments from rocky shores to the shelf to abyssal sediments (Sedlacek and Thistle, 2006; Sarmiento et al., 2017; Rossel and Martínez Arbizu, 2019). Most of the copepod species within this study share their genera with other vent environments such as the JFR and EPR. Three species of the Ectinosomatidae family were identified in this study, *Halectinosoma* sp. 1, *Halectinosoma* sp. 2 and *Halophytophilus* sp. 1. These harpacticoid genera are more typical of non-vent, sedimentary deep-sea environments (Plum et al., 2017). Differences in the copepod

community structure between snail and mussel habitats can be clearly seen in the multivariate nMDS analysis which points to discrete communities, with few species that are highly adapted to extreme vent conditions clustering together (habitat specialists). Species that are shared with ambient waters and may require higher oxygen concentrations (habitat generalists) also cluster together, a global trend that can be seen wherever mussel beds occur at hydrothermal vents (Zekely et al., 2006a; Gollner et al., 2010b).

Trophic diversity may play a vital role for ecosystem functioning in these areas. The distribution of organisms at hydrothermal vents is driven by a coupling of tolerance to extreme conditions and by the ability of the organism to make use of the primary production in these habitats, as productivity is known to partially correlate with environmental stress (Le Bris et al., 2019). The very specialised order of siphonostomatoid copepods have characteristic "siphon-like" mouth-parts that allow them to graze on bacterial mats that form on rock formations (or on snail shells) at the more extreme end of the stress gradient. The exact feeding ecology of *S. lauensis* has not been observed, but trophic interactions have been documented for *S. quadrispinosus* (Limén et al., 2008), and this species feeds predominantly on specific strains of chemoautotrophic bacteria. It has a small mouth opening of ~5 µm, whereas *Benthoxyneus speculifer* has a slightly larger mouth opening of ~20 µm and is less discriminatory, feeding on various strains of autotrophic and heterotrophic bacteria (Limén et al., 2008). The mouth opening of *S. lauensis* is ~35 µm, indicating that it may also not discriminate as much as *S. quadrispinosus*. In contrast, members of the Harpacticoida found in the mussel samples, which although an incredibly diverse group, do not have mouth parts equipped to feed on bacterial mats in this way. A possible exception is the Tisbididae family, whose mouth parts resemble the siphon-like parts of the Dirivultidae. This trophic diversity may play a vital role for the ecosystem functioning

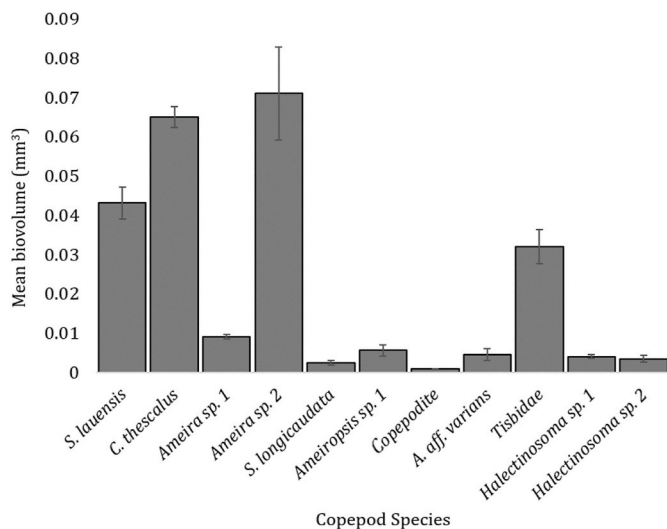
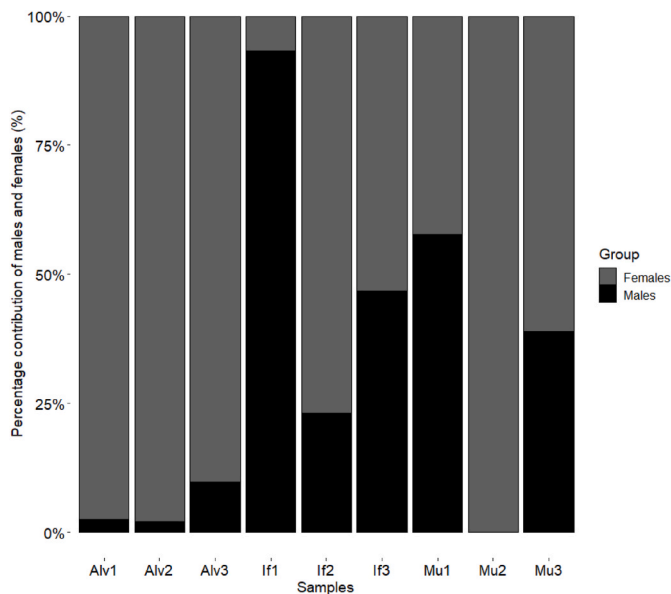


Fig. 5. Mean biovolume (mm<sup>3</sup>) of the most abundant species in the overall study that contribute the most to biomass at each of the habitats.



**Fig. 6.** Percentage of females vs. the percentage of males for *S. lauensis* in each sample *Alviniconcha* (Alv1-3), *Ifremeria* (If1-3), and *Bathymodiolus* (Mu1-3). Note: Mu2 sample included only one individual. Black bar = males, grey bar = females.

in these areas.

#### 4.3. Effects of environmental variables on diversity and community composition

According to the results of the *BIOENV* analysis, varying levels of oxygen were the best explanatory variable for community composition. However, the highest species richness (17) was found at the Mu1 sample, where intermediate oxygen concentrations were measured at  $71.09 \mu\text{mol L}^{-1}$ , potentially highlighting the idea that the highest diversity exists at intermediate stress levels, a theory that has been widely used to explain community patterns on spatial and temporal scales within the framework of patch dynamics (Kukert and Smith, 1992; Roxburgh et al., 2004). No copepods aside from the Dirivultidae were found in the higher temperature ranges, possibly as they have not developed adaptive mechanisms for temperatures above those found at the samples across the *Ifremeria* habitat. As the variables of temperature and  $\text{H}_2\text{S}/\text{HS}^-$  covaried significantly (Gartman et al., 2011), the latter was used as a proxy for the former in the results, but this relationship may vary across even small spatial scales (across different microhabitats comprised of megafauna assemblages) (Le Bris et al., 2003; Le Bris, 2006a, b) and with time at a site (Luther et al., 2008; Moore et al., 2009). The Dirivultidae have adapted to these environments in a variety of ways including the evolution of a haemoglobin protein that has a particularly high and temperature-sensitive oxygen affinity, quantified in two copepods of the Dirivultidae, *Benthoxynus spiculifer* and *Scotoecetes introrsus* (Hourdez et al., 2000; Sell, 2000, respectively), an adaptation to low-oxygen environments. The Harpacticoida reported in this study, may indeed not have adaptations that allow them to thrive in the more extreme vent environment, but their presence and diversity indicate that species may exploit the enhanced productivity encountered under vent conditions relative to the surrounding environment.

#### 4.4. Megafaunal amelioration of the physiochemical environment

The disparity between the physiochemical measurements amongst the megafauna and the faunal hole created after sampling the megafauna with the mussel-pot may be a result of megafauna ameliorating their local environment. Three megafauna species in this study contain

symbiotic chemoautotrophic, sulphur-oxidising bacteria (Desbruyères, 1994; Podowski et al., 2009, 2010; Gartman et al., 2011; Govenar, 2012; Sen et al., 2014). The snail species, here associated with the more extreme end of the spectrum, may not only act as a habitat for meiofauna, but also ameliorate the local environment by actively decreasing the concentrations of  $\text{H}_2\text{S}/\text{HS}^-$  via endosymbiotic activity. High pressure incubation experiments (Gartman et al., 2011; Beinart et al., 2015) indicated that *Ifremeria* snails containing chemosynthetic endosymbionts also release polysulfides, potentially being responsible for the observation of these sulphur species in the field. However, they noted that it was not possible to discern between sulfide detoxification mechanisms, the symbionts' metabolic activities, free-living microbes associated with the shell or abiotic reactions due to metal as being responsible for the sulphur oxidation observed during the experiments. Further research needs to be conducted to investigate the true adaptive mechanisms of the *S. lauensis*, and whether it could withstand higher temperatures and concentrations of  $\text{H}_2\text{S}/\text{HS}^-$ .

#### 4.5. Size and biomass

The life history traits of differently sized organisms and the effect of this on physiology and biochemistry has been discussed by Gollner et al. (2015) with emphasis on the contrast between macro- and meiofauna. The implications of these interspecific differences in body size are many even among the meiofauna. Larger organisms produce fewer offspring (*S. lauensis* produces a maximum of 4 eggs per reproductive cycle, Ivanenko et al., 2007, 2011). However, the few offspring produced by *S. lauensis* have not hindered its ability to exist in high densities compared to other species. The rather large lecithotrophic nauplii of dirivultids may make them more successful in terms of survival rates than the rather small planktotrophic nauplii of harpacticoids. Adult dirivultids are large and have a protective, chitinous exoskeleton, as well as biochemical adaptations to deep-sea vent environments. The smaller, fusiform harpacticoids are not restricted to this environment as they may require a smaller amount of nutrients to sustain their small sizes, available in just enough quantities at the interface between vent and non-vent environments (Gollner, 2015). Furthermore, the morphological variation in the mouthparts of the species identified, indicates a difference in the food demands with implications for variation in metabolic rate.

Copepod biomass was considerably higher among the *Alviniconcha* habitat than among the mussel beds due to the large size and high abundance of *S. lauensis*. Biomass  $10 \text{ cm}^{-2}$  encountered for snails at ABE falls within the range of that reported by Gollner et al. (2006) for tubeworm habitats for the EPR with a temperature range of  $18\text{--}23 \text{ }^\circ\text{C}$  ( $0.02\text{--}3 \text{ mg}$ ). Copepod biomass at the mussel habitat were higher at the EPR ( $2\text{--}3 \text{ mg}$ ) (Gollner et al., 2006) than at the mussel habitat at ABE.

#### 4.6. Gender niche partitioning

Females and males of the three dirivultid species identified exhibit a clear sexual dimorphism with females being considerably larger than males. For all three species sex ratios were skewed towards females, a trend encountered in other vent sites for congener species (Tsurimi et al., 2003). The latter study suggests that males face higher mortality rates, possibly dying directly after mating events and that females can fertilize several egg clutches after a single mating event. Another possibility is that males of *S. lauensis* might be less tolerant to higher levels of  $\text{H}_2\text{S}$  than females. From Alv3 to If1, there is a shift from a  $\sim 90\%$  female dominated population to one of  $>90\%$  males (Fig. 6). In the environmental data, Alv1-3 have relatively stable temperatures, but between Alv3 and If1 there is a significant decrease in  $\text{H}_2\text{S}/\text{HS}^-$  (reflected in the shift from females to males). Both males and females appear to be able to tolerate a decrease in  $\text{H}_2\text{S}$  concentrations, potentially leading to intra-specific competition for resources driving variations in the ratios of the males and females towards the less-extreme end of the stress

gradient. Senokuchi et al. (2020), however, found that populations of *Stygiopontius senokuchiae* from vents in knoll-associated calderas of the Northwest Pacific Ocean were skewed significantly towards males. Further research therefore, needs to be conducted to into sex ratios of this genus to understand patterns in gender niche partitioning.

#### 4.7. Implications for deep-sea mining and further research

Accurate diversity estimates of phyla that play important ecological roles are crucial to advising ecosystem monitoring programs and placements of marine protected areas (Leasi et al., 2018). For vents, species effort curves for copepods indicate that sampling effort is sufficient to reach an asymptote (Gollner et al., 2006). With sampling effort constantly increasing, and molecular methods being standardized and improving over the last decade, diversity estimates are constantly being revised upwards (Plum et al., 2017). However, we are still sorely lacking data on smaller sized organisms that shape much of the community structure at hydrothermal vents. Research in this field is increasing and across many studies it is now widely accepted that the impacts of deep-sea mining on benthic communities will be significant due to the predicted nature and scale of exploitation in specific locations such as the Western Pacific (Van Dover, 2014; Gollner et al., 2017; Van Dover et al., 2017; Miller et al., 2018; Niner et al., 2018).

## 5. Conclusion

This study reveals very structured copepod communities in areas of diffuse hydrothermal venting reflecting their tolerance to a stress gradient of varying temperatures and concentrations of sulfide and oxygen, that compliments the structure of the dominant megafauna species in this area. Diversity and species richness increased towards the oxygenated end of the stress gradient, with many organisms sharing genera with that from shallow coastal waters, shallow vents and peripheral vent environments resulting in very distinct copepod communities between the snail and the mussel megafauna habitats. Abundances and biomass were similar to other vent areas. The dominant species of copepod *S. lauensensis* exhibited stark gender niche partitioning to different concentrations of H<sub>2</sub>S, something not seen before for this species.

This study focuses on one single hydrothermal diffuse flow venting site within the entire Lau Basin where even at such small spatial scales, a clearly structured copepod community is encountered. Sampling effort needs to be increased in this area across many vent fields to obtain data on succession, connectivity and resilience and time series data need to be collected to investigate changes in community structure in a comparable way to that of megafaunal assemblages. There is currently no data on succession or recovery ability of meiofauna within the West Pacific vent fields, yet the latter stand as potential targets for mining companies interested in SMS deposits. More data on biodiversity and community dynamics is required for this region if policy making for deep-sea mining is to be properly informed.

### Author contributions

**Coral Diaz-Recio Lorenzo:** copepod identification, data analyses, writing of draft manuscript; **Daisy ter Bruggen:** copepod identification; **George W. Luther, III:** physiochemical analyses, revision of draft manuscript; **Amy Gartman:** physiochemical analyses, revision of draft manuscript; **Sabine Gollner:** design of the project, sample collection, help in copepod identification, revision of draft.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dsr.2021.103532>.

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