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Spatial and temporal variations in food web structure from newly-opened habitat at hydrothermal vents

Sylvie Marylène Gaudron[a, *], Sébastien Lefebvre[b], Amandine Nunes Jorge[a, c], Françoise Gaill[a, d], Florence Pradillon[a, e]

[a] Université Pierre et Marie Curie, Bâtiment A 4ème étage pièce 415, Paris VI, CNRS, UMR7138, Systématique, Adaptation, Evolution, équipe AMEX, 7 quai St Bernard, 75252 Paris cedex 05, France
[b] Université de Lille1 Sciences et Technologies, UMR CNRS 8187 LOG « Laboratoire d'Océanologie et Géosciences», Station marine de Wimereux, 28 av. Foch, 62930 Wimereux, France
[c] Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany
[d] CNRS, INEE, 3 rue Michel Ange, 75017 Paris, France
[e] IFREMER, Centre de Brest, Département des Ressources physiques et Ecosystèmes de fond de mer, BP 70, 29280 Plouzané, France

*: Corresponding author : Sylvie Marylène Gaudron, Tel.: +33 (0) 144273781 ; fax: +33 (0) 144272145 ; email address : sylvie.gaudron@snv.jussieu.fr

Abstract:

To highlight the spatio-temporal variability of the food web structure of hydrothermal vent fauna from newly-opened habitat, a series of Titanium Ring for Alvinellid Colonization devices (TRACs) was deployed at TICA site on the East Pacific Rise in 2006. This experiment was conducted for periods of 4 days, 13 days and one month and deployments were aligned along a gradient from the basaltic bottom to the vent openings. d13C values of colonists revealed a narrower range of carbon sources in proximity to vent openings in Alvinella pompejana habitat than in Tevnia jerichonana habitat, separated by a distance of four meters. This was possibly due to a spatial change in available food sources with a possible higher contribution of particulate organic matter (POM) to the siboglinid habitat compared to a higher contribution of microbial primary producers such as Epsilonproteobacteria in the alvinellid habitat. Temporal variability was also observed during experimentation in the form of a shift in either d13C and/or d15N values for A. pompejana, Lepetodrilus elevatus, dirivultid copepods and polynoid polychaetes within a one-month window showing first of all, fast tissues turnover and secondly, a possible switch in feeding strategy or food sources. Lepidonotopodium riftense and Branchinotogluma sandersi may have to alternate between detritivorous and predatory feeding strategies. In addition, through the analysis of stable isotope composition of A. pompejana and its episymbionts, we provided evidence that these attached bacteria formed part of the worms' diet during the course of these colonization experiments.

Highlights

► We analyzed the food web structure of vent fauna within colonization experiments. ► We demonstrated the small spatial and small temporal variability of the food web structure. ► We demonstrated the switch of feeding strategies for some metazoans. ► We demonstrated the change of food sources for nutritional strategy of some metazoans. ► Episymbionts of *Alvinella pompejana* may be part of their diet.

Keywords: Trophic relationships ; Food web structure ; Hydrothermal vent ; East Pacific Rise ; Stable isotopes ; Colonization experiment ; TRACs ; *Alvinella pompejana* ; Epibiosis

1 INTRODUCTION

Hydrothermal vents are ecosystems where primary producers are chemolithoautotrophic microorganisms that take advantage of the mixing interface between seawater and vent fluids. These microbes fix inorganic carbon through the oxidation of reduced compounds, into organic carbon, at the base of the food web (Jannasch, 1985; Karl, 1995; Childress and Fisher, 1992). In contrast to the paucity of conspicuous life forms in the deep ocean, hydrothermal vents harbor a flourishing biomass of megafauna where one or two species are visually dominant. Depending on vent geographic location and local environmental factors, these dominant species may belong to polychaete tubeworms, bathymodiolid mussels, vesicomyid clams, alvinocarid shrimps, and/or provannid gastropods. Many of them are foundation species that create habitat or modify the local density and diversity of macrofaunal invertebrates (Govenar, 2010). Foundation species also often harbor chemoautotrophic symbiotic bacteria that are their primary source of nutrition. Hydrothermal vents are very unstable in terms of abiotic factors such as the chemical composition of the emitted fluid (sulfide, methane or hydrogen) and temperature (Luther et al., 2001; Le Bris et al., 2006). The spatial and temporal variability of abiotic factors not only dictates the structure of metazoan communities (Tunnicliffe, 1991; Sarrazin et al., 1999; Cuvelier et al., 2009) but also has an indirect impact on carbon fluxes within the hydrothermal vent food web (Limén et al., 2007; De Busserolles et al., 2009; Govenar, 2012). Deep-sea hydrothermal vents are similar to intertidal zones in terms of habitat heterogeneity, where patchiness of animal communities may occur at the decimeter scale (Levesque et al., 2006; Dubois et al., 2007). They typically display faunal zonation along environmental gradients related to the distance from vent openings (Sarrazin and Juniper, 1999; Limén et al., 2007). The metazoan species *Alvinella pompejana* lives in closer proximity to vent openings being both more thermotolerant and adapted to high environmental sulfide concentrations (Le Bris and Gaill, 2007), when compared to species further away from the vent openings such as *Riftia pachyptila* (Tunnicliffe, 1991; Van Dover, 2000; Nees et al., 2009).

Several food sources are at the base of the food web of hydrothermal vents. A percentage of particulate organic matter (POM) is known to contribute to the diets of non-symbiotic hydrothermal vent fauna (Levesque et al., 2005, 2006; Limén et al., 2007). It is composed of endogenous sources including a mixture of detritus from decaying bodies, mucus and heterotrophic microbial cells and exogenous sources such as small fractions of photosynthetically-derived surface material (diatoms and coccolithophorids) and associated bacteria (Levesque et al., 2005). At Juan de Fuca Ridge, in the Northeast Pacific, stable carbon isotopic composition ($\delta^{13}C$) of POM vary at the scale of a single sulfide edifice, which reflects an increasing proportion of exogenous sources of organic matter with increasing distance from the vent fluid emission (Limén et al., 2007). Other significant sources of organic matter are provided by free-living microbes and symbiotic bacteria (Bergquist et al., 2007; Govenar, 2012). For instance, *Epsilonproteobacteria* (free-living microbes) are considered to be key players in the cycling of carbon, nitrogen and sulfur at hydrothermal vents on the East Pacific Rise (EPR) (Campbell et al., 2006; Sievert et al., 2009; Sievert and Vetriani, 2012). As a consequence of the variability in available food sources at increasing distance from fluid emissions at the scale of a single vent sulfide edifice, food web structures may also display significant differences at similar spatial scale. Despite a number of studies attempting to decipher trophic relationships at hydrothermal vents using the stable isotope technique (e.g. Van Dover and Fry, 1989; Van Dover and Fry, 1994; Fisher et al., 1994; Vereshchaka et al., 2000; Colaço et al., 2002; Levesque et al., 2003, 2006; Limén et al., 2007; Debusserolles et al., 2009), complex feeding relationships among a single entire animal community retrieved with a “bushmaster” sampling device were only recently elucidated at a diffuse flow site of Juan de Fuca Ridge in the Northeast Pacific (Bergquist et al., 2007). Levesque et al. (2006) simultaneously studied temporal and spatial trends in stable isotope compositions of 10 representative invertebrates’ species and revealed that species stable isotopic ratios and food web structures were constrained by the faunal community structure over yearly time scales and tens of meters to kilometers spatial scales. On a much finer scale (decimeters), Limén et

al. (2007) and De Busserolles et al. (2009) evidenced the influence of local environmental conditions that may shape small scale variations of the food sources, of the faunal stable isotopic ratios and of the food web structure.

In situ colonization experiments have been carried out for over two decades at hydrothermal vents using polycarbonate plates, basalt rocks, sponges or titanium rings (Van Dover et al., 1988; Shank et al., 1998; Taylor et al., 1999; Mullineaux et al., 1998, 2003; Pradillon et al., 2005, 2009; Kelly et al., 2007; Kelly and Metaxas, 2008). **T**itanium **R**ing for **A**lvinellid **C**olonization (**TRAC**s) deployed near vent openings at hydrothermal vents in the Pacific (East Pacific Rise 9°N and13°N) demonstrated that the alvinellid polychaete *Alvinella pompejana* was a pioneer metazoan species among assemblages that colonized TRACs on smoker sulfide edifices, after the initial colonization of filamentous bacteria (Taylor et al., 1999; Alain et al., 2004; Pradillon et al., 2005, 2009). Alvinellid worms secrete tubes or mucus on the surfaces they colonize, and may locally modify flow patterns, fluid emission, mineral precipitation and the degree of hydrothermal mixing with seawater, allowing establishment of other species exhibiting a lower tolerance to severe hydrothermal conditions (Juniper et al., 1992; Juniper and Martineu, 1995; Sarrazin and Juniper, 1999; Zbinden et al., 2003; Le Bris et al., 2005; Pradillon et al., 2009). Colonization of new active hydrothermal edifices by metazoan species generally mostly occurs through larval dispersal and recruitment (Lutz et al., 1984). However, newly-available surfaces in TRAC experiments deployed by Pradillon et al. (2005) over variable time intervals between 1995 and 1999 at EPR 9°50'N and 13°N were mainly colonized by post-larval stages, juveniles and adults. The occurrence of large sized *A. pompejana* on TRACs deployed for only a few days suggested that those individuals migrated by secreting new tubes from adjacent parts of the sulfide edifice, rather than recruiting as larvae. Pradillon et al. (2009) showed that following TRAC deployments on active sulfide edifices, complex structure made of mineral precipitation and alvinellid tubes quickly form and tend to buffer the sharp centimeter scale temperature gradients, thus allowing the development of a more diverse faunal communities within a

few days. The authors proposed that the slight differences observed in the community structures of assemblages retrieved from different TRACs reflected local environmental conditions. Trophic relationships and resources partitioning may also significantly affect the development of these assemblages, but they were not investigated in that study.

Here, we are looking at the variability of the food web structure in the early steps of faunal assemblage formation using stable isotope analyses in order to highlight the possible small spatial scale (few meters) and temporal scale (few days to a month) variability of samples collected on and around hydrothermal vent edifices. We conducted new *in situ* colonization experiments by deploying 3 successive series of TRACs along a spatial gradient from a vent opening at the EPR 9°50'N. Closest to the vent opening, were TRACs deployed among alvinellids, where we expected the quick formation of a complex mineral-tube structure accompanied by the development of the faunal assemblage. The two other habitats selected were at the basis of *Tevnia jerichonana* tubeworms, and on bare basaltic seafloor within a few meters from the alvinellid deployment. In the sibloglinid habitat, although rather high temperature might be expected, the quick formation of a mineral-tube matrix is not expected, which may result in a completely different faunal assemblages development, perhaps much slower. Experiments were conducted for three periods: 4 days, 13 days and one month. The questions to be addressed in this paper are: 1) Are the stable isotopic compositions of some target non-symbiotic invertebrates recovered from colonization experiments from different habitats on a vent variable with deployment duration (4d, 13d and 29-33d)? 2) Do food web structures at vents vary with space and time? 3) Is *Alvinella pompejana* able to feed on its episymbiotic bacteria in experimental conditions?

2 MATERIAL AND METHODS

2.1 Study site, TRACs and fauna

Our study site (Fig.1) was a basalt hosted vent system at 9°50'N on the East Pacific Rise (EPR), where a volcanic eruption occurred in winter 2005-2006, which covered many of the previously established vent animal communities (Soule et al., 2007; Bennet et al., 2011). In this study, colonization experiments were carried out a few months after the eruption. At that time, animal communities were re-establishing themselves around new vigorous vents and large aggregates of symbiotic *Tevnia jerichonana* tubeworms, up to 30 cm long (Nees et al., 2009) as well as alvinellid polychaetes colonies were observed. Series of three colonization devices (**TRAC**s for **T**itanium **R**ing for **A**lvinellid **C**olonization) were deployed on each occasion for incremental time periods (4 days, 13 days and 29-33 days) between November and December 2006 (Table 1) during the two oceanographic cruises LADDER 1 and 2 at two active sites (Fig.1). For each series (representing one deployment interval), three deployment locations were selected: one on alvinellid colonies, one near *T. jerichonana* tubeworms, and one on the bare basalt out of venting influence. Eight TRAC deployments (Fig.2; Table 1) were carried out at the TICA site (9°50'24''N, 104°17'30''W), where active venting occurred along the eastern wall of the axial graben. Exposed surfaces of basalts in vigorous diffuse flows were densely colonized by *T. jerichonana* siboglinid tubeworms, and were adjacent to sulfide flanges with more focused hot fluid emissions and assemblage of alvinellids. Two TRACs were deployed on the sulfide flanges covered by alvinellids for 4 and 13 days, three TRACs were deployed at the base of *T. jerichonana* siboglinid tubeworms for 4, 13 and 29 days, and three TRACs were deployed a few meters away, on fresh basalts for 4, 13 and 29 days (Fig.2; Table 1). Due to dive logistics constraints, our one-month TRAC deployment on alvinellids was not at the TICA site but in a similar environment at the Bio_9 site (9°50'18''N, 104°17'32''W) (Fig. 1; Table 1). Bio_9 was a large black smoker complex of more than 20 spires hosting alvinellid polychaetes located about 150 m from the TICA site (Fig.1). Temperature was measured before deployment of each TRAC, and areas with temperatures below 20 °C were selected. The TRACs (15cm in diameter and 10cm in height) were hollow cylinders that mimicked a newly-

opened surface when deployed on an active hydrothermal edifice. They had holes in their sides to enable circulation of vent fluids and contained internal spokes to enable anchoring of alvinellid tubes and associated fauna (Pradillon et al., 2005, 2009). Each TRAC was equipped with MICREL autonomous probes that semi-continuously recorded the temperature inside the device during the *in situ* deployment (Table 1). Deployment and recovery of TRACs were performed by the manned submersible Alvin (Woods Hole Oceanographic Institution, USA) (Table 1). At recovery, devices were placed in a hermetically-sealed box to avoid washing and mixing during the ascent.

After recovery, the whole assemblage recovered on each TRAC was directly fixed on board in 10% formalin in filtered seawater, thus keeping the three-dimensional structure of the faunal community (Zbinden et al., 2003). Later, in the laboratory, samples were transferred to 70% ethanol. Organisms were sorted under a dissecting microscope and identified to the lowest taxonomic level possible using morphological characters, published species descriptions (Desbruyères et al., 2006) and personal advice on polynoid polychaetes (Daniel Desbruyères, IFREMER, pers. communication). Twenty-four taxa were identified in total (data not shown). Specimens of each taxa were counted and consequently the density of each taxa within TRAC (volume = 1.77 dm^3), was calculated (Table 2). Feeding guilds (bacterivore, detritivore or predator) were assigned based upon known feeding biology from the literature where available and on the model of Bergquist et al. (2007) (Table 2). Only dominant taxa were used for stable isotopes analyses (Table 2).

2.2 Sample preparation for stable isotope analyses

Seven invertebrate taxa recovered from TRACs deployed on the alvinellid and the siboglinid habitats were analyzed. Due to the very few colonists on TRACs deployed on bare basalt (Table 2), no stable isotopes analyses (SIA) were carried out on these samples. For large animals such as the polychaete *Alvinella pompejana*, SIA were conducted on tissues of each specimen separately (n = 5 for each TRAC). We analyzed the body wall removing the digestive tract and the episymbiotic

bacteria that were attached to the dorsal surface. These episymbionts were removed under a dissecting microscope using forceps. For the gastropod *Lepetodrilus elevatus*, tissues separated from the shell of 20 specimens were pooled to obtain enough material for SIA. Similarly, SIA were conducted on pools of 3 specimens of the polychaetes *Lepidonotopodium riftense* and *Branchinotogluma hessleri*, on pools of 2 specimens of the polychaete *B. sandersi*, on pools of 20 specimens of *Ventiella sulfuris* and on pools of 100 specimens of dirivultid copepods with similar morphotypes. Tissues were rinsed with distilled water, oven-dried at 60 °C for 48 h and ground to powder with a mortar and pestle. To avoid significant changes in $\delta^{15}N$ isotopic composition, no HCl was used to remove carbonates (Kaehler and Pakhomov, 2001). Preservation in formaldehyde and ethanol can lead to bias in the SIA, as ethanol may increase $\delta^{13}C$ while formaldehyde may decrease it (Kaehler and Pakhomov, 2001). However, previous SIA performed on the same type of hydrothermal vent metazoans that were fixed in formaldehyde and later transferred to ethanol, consistently showed no significant differences from frozen samples (Bergquist et al., 2007; De Busserolles et al., 2009). Finally, we did not perform any lipid treatment since the C:N ratios of all organisms studied for SIA were between 3 and 4 (Post et al., 2007).

For each species, samples were prepared for analyses in tin combustion capsules (1 mg ± 0.1) and analyzed using a CHN elemental analyzer (EuroVector, Milan, Italy). The resultant gas was analyzed online in an isotope ratio mass spectrometer (IRMS) (GV IsoPrime, UK) to determine carbon and nitrogen stable isotope ratios. Stable isotopic data are expressed in permil (‰), and quantify the relative difference between the rare-to-common isotope ratio in a sample and the corresponding conventional standard, defined as Pee Dee Belemnite (PDB) for carbon ratios, and atmospheric N_2 for nitrogen ratios, according to the following equation:

$$\delta(X) = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] * 1000$$

where X (‰) is ^{13}C or ^{15}N abundance and R is the ^{13}C:^{12}C or ^{15}N:^{14}N ratios. The internal standards used were USGS 40 ($\delta^{13}C$ = -26.8‰; $\delta^{15}N$= -4.5‰) and USGS 41 ($\delta^{13}C$ = 37.6‰; $\delta^{15}N$ = 47.6‰) from the International Atomic Energy Agency. The typical analytical precision was ± 0.05‰ for carbon and ± 0.12‰ for nitrogen.

2.3 Trophic position

We used the formula proposed by Post (2002) to estimate the trophic position (TP) of the metazoans that colonized TRACs, using the average $\delta^{15}N$ ratio of *Alvinella pompejana* episymbionts as a trophic baseline. Episymbionts of *A. pompejana* are mostly dominated by *Epsilonproteobacteria* and stable isotopic ratios ($\delta^{13}C = -12.4$‰; $\delta^{15}N = 4.8$) are in the range of stable isotopic ratios of *Epsilonproteobacteria* from EPR 9°50N (Campbell et al., 2003), viewed as free-living bacteria and primary producers at diffuse-flow hydrothermal vents (Bergquist et al., 2007; Govenar, 2012). We have adjusted the trophic fractionation to 3.3 ‰ in $\delta^{15}N$ for microbial diet and 1.4‰ in $\delta^{15}N$ for invertebrate diets (predators) according to Bergquist et al. (2007):

$$TP = (\delta^{15}N_{organism} - \delta^{15}N_{epibionts})/3.3+1 \text{ or } TP = (\delta^{15}N_{organism} - \delta^{15}N_{epibionts})/1.4+1$$

2.4 Statistical analyses

To examine the variation in food sources between taxa recovered from deployments within the alvinellid habitat compared to the taxa recovered from deployments within the siboglinid habitat, we used a Bartlett's test to test differences between variances of $\delta^{13}C$ values (MINITAB version 15). To determine whether $\delta^{13}C$ ratios of *Alvinella pompejana* differ between colonization experiment duration (4d, 13d and 33d) within the alvinellid habitats, a one-way ANOVA was carried out and significant differences were assessed by using a post-hoc Tukey test (MINITAB version 15).

3 RESULTS

3.1 Temperature and deployment duration

Mean temperatures that occurred within TRAC deployed on alvinellids for 4 days at the TICA site and for 33 days at the BIO_9 site were in the same range (8.6 °C ± 2.7; 6.3 °C ± 1.9 and 8.7 °C ± 2.4 respectively; Table 1). During the 13-day deployment on alvinellids at the TICA site, both temperature probes exhibited a steep and severe temperature increase, far beyond the maximum temperature of 20 °C initially chosen for our deployment (Fig.3c). Temperature rose over 150 °C and was probably much higher, but precise measurement of maximum temperature was not obtained because it exceeded the accuracy limit of the probes (Fig.3c). A small sulfide spire, about 10cm high, grew within the TRAC during the 13-day deployment interval. The tip of the probes was very close to the growing spire, and was probably bathed by high temperature fluid expelled from the spire. Temperature was highly variable and changed within minutes in this alvinellid habitat (Fig.3c). Within the siboglinid habitat, the same temporal trend was observed, but the amplitude of variation was reduced (Fig.3b). Mean temperatures that occurred within TRACs deployed for 4 days and 29 days at the TICA site among siboglinid habitat were in the same range (~5 °C), but less than those mean temperatures recorded during deployment within alvinellid habitats for the same duration (Table 1). However, as was the case in the 13-day deployment among alvinellid habitat (though less extreme), high temperatures in TRAC deployed within the siboglinid habitat were also recorded (up to ~29 °C) giving a mean temperature of 10.5 °C ± 4.9 (Fig.3b; Table 1). By way of a control, TRACs deployed on bare basalt had more or less stable temperature throughout the different deployment duration (4 days, 13 days and 29 days), with a mean temperature ~2 °C similar to ambient deep-sea temperature (Fig.3a; Table 1).

3.2 Stable isotopes values of carbon and nitrogen in alvinellid habitat

In the alvinellid habitat (Fig. 4a), the amphipod *Ventiella sulfuris* and the gastropod *Lepetodrilus elevatus* yielded lighter $\delta^{13}C$ (-13.3/-13.1‰ and -13.2‰ respectively) while the heavier $\delta^{13}C$ was recorded for the polychaete *Alvinella pompejana* (-10.6‰ ± 0.9‰) and dirivultid copepods (-10.6‰), after 4 days of deployment. Both latter taxa exhibited the same range of $\delta^{15}N$ isotopic ratios (6.5‰) and became more $\delta^{13}C$ depleted after 13 days (-11.9‰ ± 0.3‰; -12.3‰ respectively) compared to the 4-day deployment (Fig. 4a). Dirivultid copepods were absent from TRAC deployed on alvinellid habitat in Bio_9 (Table 2). Significant differences in $\delta^{13}C$ of *A. pompejana* were tested between colonization experiment duration (4d, 13d and 33d) ($F_{(1,2)} = 7.47$; $p < 0.01$). A post-hoc Tukey test showed that $\delta^{13}C$ values of *A. pompejana* recovered from the 4-day deployment are different from those of the 13-day deployment ($p < 0.05$) (Fig.4a).

One of the possible primary producers, the episymbionts of *A. pompejana* displayed the lighter $\delta^{15}N$ values (4.8-5.1‰) and were more $\delta^{13}C$ depleted (-12.1 to -12.5‰) than to their host (data seen previously) (Fig. 4a). Thus after 4 days of deployment, trophic fractionation (Δ hereafter) between *A. pompejana* and its episymbionts, was 2.2‰ for $\delta^{13}C$ and 1.5‰ for $\delta^{15}N$ while Δ of $\delta^{13}C$ were 0.3‰ and 0.8‰ and Δ of $\delta^{15}N$ were 1.4‰ and 1.8‰ after 13- and 33-day deployments respectively (Fig.4a).

3.3 Stable isotopes values of carbon and nitrogen in siboglinid habitat

A temporal variability in stable isotopic composition of target invertebrates was observed in the *Tevnia jerichonana* habitat during the course of the experiment (Fig. 4b). The gastropod *Lepetodrilus elevatus* yielded lighter $\delta^{13}C$ values after 13 days (-14.2‰) and 29 days (-14.5‰) of deployments compared to the 4-day deployment (-11.5‰). However, the $\delta^{15}N$ isotopic values (8.2-8.7‰) of this species did not change in any of the three temporal series of colonization experiments (Fig. 4b). The heavier $\delta^{15}N$ values were measured in the two polynoid polychaetes *Branchinotogluma hessleri* and *B. sandersi* after 13- and 29-day deployments (Fig. 4b; 12.1 and

12.3‰ respectively). The polynoid *Lepidonotopodium riftense* was highly $\delta^{13}C$ depleted compared to the rest of the taxa in the three temporal colonization experiments and also displayed a very wide range of $\delta^{15}N$ values (from 6.7 to 10.8‰) (Fig. 4b). Dirivultid copepods collected after 4 days of deployment (Fig. 4b) had the lighter value of $\delta^{15}N$ (4.1‰) among the metazoans and prokaryotes analyzed in this study.

3.4 Spatial variability of stable isotope ratios

Overall, $\delta^{13}C$ values of taxa recovered in TRACs deployed within the *Alvinella pompejana* habitat were significantly more homogeneous (-13.3 to -10.1‰) than those recovered in the *Tevnia jerichonana* habitat (-15.6 to -10.5‰; Bartlett's test $F = 0.37$; $p < 0.05$). Both dirivultid copepods and the limpet *Lepetodrilus elevatus* were more $\delta^{13}C$ depleted in the siboglinid habitat than in the alvinellid habitat (Fig. 4a, b). Stable isotope values of the amphipod *Ventiella sulfuris* were very similar ($\delta^{13}C$: -13.1 to -13.7‰; $\delta^{15}N$: 8.3 to 8.6‰) in specimens collected in the six TRACs deployed in the two different habitats (alvinellid and siboglinid habitats) over time and also in the different sites (Fig. 4a, b).

3.5 Food web structures

Three main trophic positions (TP) emerged from the Table 3 giving potentially four trophic levels including primary producer, the episymbionts (attached bacteria of *Alvinella pompejana*) as the first trophic level (TP ranging ~1). The first group of primary consumers (TP ranging between ~1 and ~2) including *A. pompejana* and dirivultid copepods was seen as bacterivores. A second group of primary consumers (TP ranging between ~2 and ~3) including *Ventiella sulfuris* and the limpet *Lepetodrilus elevatus* was seen as detritivores. A third group involving the secondary consumers (TP ranging above 3), was seen to comprise of predators including the three polynoid polychaetes. The two scale worms *Branchinotogluma sandersi* and *Lepidonotopodium riftense* may

switch their diet and become detritivores (Table 3). Within the alvinellid habitat, no changes of feeding guilds occurred during the course of the experiments between the 4-day, 13-day (TICA site) and also the 33-day experiments (Bio_9 site). However, within the siboglinid habitat, two species switched their feeding strategy in both directions from detritivore to predator from the 4-day to the 29-day experiments. The trophic position of dirivultid copepods had a value lower than the trophic baseline in the 4-day experiments. For the seven invertebrate taxa encountered in any TRACs or habitats, general mean stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ with respective standard deviation are reported in Table 4. Standard deviations in $\delta^{13}C$ of *B. sandersi*, *L. riftense*, *B. hessleri*, *V. sulfuris* and *A. pompejana* were less than 1.0 (between 0.3 and 0.9) compared to those in $\delta^{13}C$ of *L. elevatus* and the dirivultid copepods that are higher than 1.0. (SD = 1.4).

4 DISCUSSION

Our colonization experiments were conducted at 9°50'N at the East Pacific Rise on vigorous vents right after a volcanic eruption, when the fauna was still in the early stages of colonization as evidenced by the presence of the siboglinid *Tevnia jerichonana*. This sessile chemosynthetic species is generally one of the first to colonize new diffuse-flow vents, and is usually replaced at a later successional stage by the siboglinid *Riftia pachyptila* (Shank et al., 1998; Govenar, 2010). Hydrothermal vents at EPR 9°50'N in November/December 2006 appeared to be at a similar successional stage to the one described by Shank et al. (1998), 10 months after the 1991 eruption at this site (Bennet et al., 2011).

4.1 Variation of the food sources and links to temperature

The Bartlett test of homoscedasticity revealed significantly larger variance in the carbon stable isotope ratios of taxa collected within TRACs deployed in *Tevnia jerichonana* habitat (Fig.4b) than those deployed in *Alvinella pompejana* habitat (Fig.4a). This might indicate a greater diversity

of food sources with different isotopic ratios in the former habitat. At hydrothermal vents, chemosynthetic primary producers are diverse both phylogenetically and metabolically (Sievert and Vetriani, 2012) but little is known on the relative contribution of these groups to the diets of primary consumers (Govenar, 2012). Though somewhat dependent on the colonization deployment duration (4d, 13d and 29/33d), generally average temperature was highest within TRACs deployed on the alvinellid habitat when compared to the siboglinid habitat (Table 1). A number of factors may co-vary with temperature at hydrothermal vents including hydrogen sulfide and pH (Le Bris et al., 2006; Gollner et al., 2010). For example, within *T. jerichonana* habitat at the TICA site, Nees et al. (2009) formerly recorded for the same range of temperatures (2-30°C) as those recorded for the 2-week colonization experiments in siboglinid habitat (Table 1), some sulfide (S_{free}) concentrations up to 549 µm and comparatively low concentrations of O_2 (mean = 27.0 µM). Several sulfur-oxidizing chemoautotrophic bacteria (*Epsilonproteobacteria*, *Aquifales* and *Gammaproteobacteria*) may be present, but as temperatures (and certainly sulfide and oxygen concentrations) were different between TRACs deployed in alvinellid and sibloglinid habitats, it is likely that any variation in the distribution of chemoautotrophic organisms is dependent upon bacteria-specific temperature regimes (Sievert and Vetriani, 2012). The spatial heterogeneity in stable isotopic ratios within TRACs may be due to these *in situ* populations of free-living microorganisms with heterogeneous carbon isotopic compositions that are microhabitat dependent (Van Dover and Fry, 1994). For example, the $\delta^{13}C$ values of bacterivorous dirivultid copepods recovered from TRACs deployed in the siboglinid habitat were lighter than those recovered from TRACs deployed in the alvinellid habitat. First of all, this could reveal that the bacteria that contribute most to the diet of specimens collected within TRACs in sibloginid habitat after 4 days and 13 days of deployments differ from those that contribute most to the diet of specimens collected within TRACs in alvinellid habitat after 4 days and 13 days of deployments. Alternatively stable isotope values of bacteria in these two particular habitats, which are only separated by 4 meters, may differ due to variability in the chemical

367 microenvironment even if the diversity and consumption ratios of different bacteria available for the
368 consumers is the same. This fact was observed by De Busserolles et al. (2009) in the Tour Eiffel
369 edifice at Mid-Atlantic ridge. Finally, there may be a notable contribution to the specimens' diet
370 from Particulate Organic Matter (POM) within the siboglinid habitat, resulting in much depleted
371 $\delta^{13}C$ values in tissues of consumers collected in this habitat, when compared to those deployed in the
372 alvinellid habitat (shift to the left in Fig.4b). Episymbiotic and free chemolithoautotrophic bacteria
373 may be the main food sources in the alvinellid habitat resulting in heavier carbon stable isotopes
374 ratios in this study. *Epsilonproteobacteria* are known to be the dominant free chemolithoautotrophic
375 bacteria on vent edifices at the 9°N EPR (Sievert et al., 2009; Sievert and Vetriani, 2012) and use the
376 reverse tricarboxylic acid (rTCA) cycle as a carbon fixation pathway (Campbell et al., 2006),
377 resulting in heavier $\delta^{13}C$ values between -12 and -8‰. This scenario is in line with the work of
378 Limén et al. (2007) who demonstrated that the further away from the flow the organisms were
379 sampled, the greater the role of POM in their diet, resulting in depleted $\delta^{13}C$ values compared to
380 organisms located closed to the fluid emission. As the metazoans' diet was dominated by microbes,
381 their $\delta^{13}C$ values were heavier.

382

383 **4.2 Variation of the diet of the primary consumers**

384 *4.2.1 Bacterivores*

385 Within a one-month window and at a distance of only 4 meters from a given sulfide edifice,
386 stable isotope values for a given taxa displayed mostly visually (Fig.4 a, b) or/and significantly
387 variability within the colonization experiments. The variability of both $\delta^{13}C$ and $\delta^{15}N$ reported in
388 different studies are listed in Table 4. In our experiments, we highlighted the temporal variability
389 (within 13 days to one month) of stable isotope ratios for two bacterivorous taxa, *Alvinella*
390 *pompejana* and dirivultid copepods. After the 2-week at the TICA site and the 1-month at the Bio_9
391 colonization experiments (Table 1), depletion in $\delta^{13}C$ was observed compared to the initial 4-day

392 experiments, reflecting an alteration in carbon sources during the course of both colonization
393 experiments (Fig. 4a). In these two TRACs (13d and 33d), a micro-environment may have
394 established. According to Taylor et al. (1999) and Alain et al. (2004), the succession of different
395 populations of microbes ultimately formed visible mats covering TRACs deployed at EPR within
396 which *Epsilonproteobacteria* were identified as being the first pioneer and dominant microbes
397 among other phylotypes. Visible mats were observed during the recovery of the TRACs, both on
398 alvinellid habitats (highly covered) and on sibloglinid habitats (sparsely covered) (Table 1).

399 *Epsilonproteobacteria* are also the dominant phylotype known to form a dense epibiosis layer
400 on the dorsal surface of *A. pompejana* (Le Bris and Gaill, 2010). These bacteria primarily use the
401 rTCA cycle as a carbon fixation pathway and at 9°50N EPR**,** $\delta^{13}C$ and $\delta^{15}N$ values are between -
402 12‰ and -8‰ and 0 to 4 ‰ respectively (Campbell et al., 2003). A shift in the diet of the
403 bacterivorous *A. pompejana* and dirivultid copepods from the 4-day experiment to the 13- and 33-
404 day experiments, could represent a change in the bacterial community within TRACs compared to
405 natural habitat. This suggests a fast tissue turnover rate in these two taxa. These two bacterivores
406 also share almost identical $\delta^{13}C$ and $\delta^{15}N$ values, indicating that they may share the same trophic
407 niche and may be in competition for the same food resource (chemoautotrophic bacteria). This would
408 provide an explanation for the decrease in density of copepods in the 2-week colonization
409 experiment and their disappearance after 1-month within alvinellid habitat (Table 2). Within our
410 study, we can exclude the possibility that *A. pompejana* may feed on dirivultid copepods, such as is
411 seen with paralvinellids *Paralvinella sulfincola* and *P. palmiformis* scavenging on the dirivultid
412 copepod *Stygiopontius quadrispinosus* in Northeast Pacific (Limén et al., 2008), based both upon
413 stable nitrogen and carbon isotopic values. In the siboglinid habitat, stable nitrogen and carbon
414 isotopic values of dirivultid copepods recovered after 4 days and 13 days within colonization
415 experiments, were very different, especially for the $\delta^{15}N$ (Fig.4b). In table 3, the trophic position was
416 lower than the trophic baseline for the dirivultid copepods sampled within the 4-day colonization

417 experiments, reflecting the diet that they consumed in their natural habitat. The change in $\delta^{15}N$ after
418 13 days of deployment may highlight a change in feeding strategy of these dirivultid copepods.

419

420 *4.2.2 Detritivores*

421 Other marine invertebrates such as the amphipod *Ventiella sulfuris* and the gastropod
422 *Lepetodrilus elevatus* may also feed on free-living micro-organisms. *Lepetodrilus sp.* are generally
423 considered as bacterivores (Tables 2, 4) but are also thought to employ suspension and/or deposit
424 feeding with epibiotic bacteria (*Gammaproteobacteria*) on its gills being an alternative nutritional
425 resource (Goffredi et al., 2004; Levesque et al., 2006; Bates, 2007). In our experiments $\delta^{13}C$ values
426 of these two bacterivorous species were lighter when compared to $\delta^{13}C$ values of the two other
427 bacterivorous species, *Alvinella pompejana* and dirivultid copepods, and were subsequently largely
428 $\delta^{13}C$ depleted in comparison to *Epsilonproteobacteria* at EPR 9°50'N (Campbell et al., 2003).
429 Therefore the chemoautotrophic bacteria cannot be the only carbon source for the gastropod or the
430 amphipod, as in this case, enrichment in $\delta^{13}C$ would be expected (trophic shift to the right in Figs.
431 4a, b). These two species may actually feed on *Epsilonproteobacteria* present within TRACs but also
432 on another type of food that is less rich in $\delta^{13}C$ such as POM, which is a mixture of bacteria and
433 decomposed organic matter. Limén et al. (2007), using a two-source mixing model (Phillips and
434 Gregg, 2003), demonstrated that POM and chemoautotrophic bacteria accounted for 40% and 60%
435 respectively of the diet of the limpet *L. fucensis*, collected in an intermediate zone of a vent sulfide
436 edifice located on Juan de Fuca Ridge. At this lower flow regime, $\delta^{13}C$ values of POM ranged from -
437 18.3 to -19.3‰ and $\delta^{15}N$ from 4.6 to 7.4 ‰ (Limén et al., 2007). A third food source,
438 *Gammaproteobacteria*, was observed in *L. fucensis* within the lamellae of its gills (Bates, 2007)
439 leading to different carbon and nitrogen isotopic ratios. Regarding the amphipod, Corbari et al.
440 (2012) found cuticles and setae of *A. pompejana* and *Epsilonproteobacteria* within the digestive tract
441 of *V. sulfuris* sampled during similar periods and sites to those in our study. These last authors

suggested that the amphipod may actually feed on the episymbionts of the alvinellid worm. However our stable isotopic data do not sustain this hypothesis as $\delta^{13}C$ of *V. sulfuris* would have been more enriched and a characteristic shift to the right would have been observed compared to the episymbionts of *A. pompejana* (Fig.4a). Both the limpet and the amphipod may have a mixed diet (Figs. 4a, b) and can be regarded both as bacterivores and/or detritivores (Tables 2, 3).

4.3 Variation in the diet of secondary consumers

Despite the fact that we cannot completely rule out the influence of extraneous sources of food upon the organisms we studied within TRACs, as the mobile species such as the errant polynoid polychaetes can prey on items outside the TRACs, we can provide a glance into possible predator-prey relationships based on the calculation of the shifts of 1‰ in $\delta^{13}C$ and of 3-4‰ in $\delta^{15}N$ (De Niro and Epstein, 1978, 1981; Minagawa and Wada, 1984) or 1.4‰ for $\delta^{15}N$ (Bergquist et al., 2007; De Busserolles et al., 2009). For instance, the carnivorous polynoid polychaete *Branchinotogluma hessleri* recovered after 1 month within the alvinellid habitat (Table 1) may have fed on both *Ventiella sulfuris* and *Lepetodrilus elevatus*. In TRACs deployed in the siboglinid habitat, we were able to identify three potential predators (Table 3): the three polynoid polychaetes *B. hessleri*, *B. sandersi* and *Lepidonotopodium riftense*. These three species did not have the same $\delta^{13}C$ isotopic values and therefore may either hunt on different types of prey or share the same range of prey but with differing magnitudes of reliance on each prey species. In the one-month experiment deployed in the siboglinid habitat (Table 1), $\delta^{15}N$ values of the polynoid *B. sandersi* were heavier than those recorded in the 4-day experiment (Fig.4b), possibly due to the increase in the number of species within the TRAC, which increased the complexity of the food web and the number of trophic positions in a classical pyramidal food web. In this short-term deployment, $\delta^{15}N$ values of *B. sandersi* reflect the nutritional resources on which the worms depend in their natural habitat (siboglinid habitat), which after calculation of their trophic position is most related to a detritical diet

(Table 3). However, the $\delta^{13}C$ of *B. sandersi* is quite enriched compared to detritical organic matter (POM) and this could indicate that this polychaete worm was preying or scavenging on dead or alive *Tevnia jerichonana* branchial plume tissue, which would explain the empty tubes of this siboglinid tubeworm found in this TRAC (data not shown). Unfortunately, we do not have any isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) of this siboglinid tubeworm from the literature to compare to our data. Bergquist et al. (2007) observed some scale worms such as *L. piscesae* feeding on the siboglinid *Ridgeia piscesae* ($\delta^{13}C$= -12.5 to -11.0 ‰ and $\delta^{15}N$= -1.7 to 2.5 ‰; Levesque et al., 2006). In this study, *B. sandersi* became the top predator after one month (Table 3) and thus may have consumed a mixed diet of different species possibly including amphipods (Fig.4b). The other top predator in the 2-week experiment in the siboglinid habitat (Table 1) was *B. hessleri*, which may prey on *V. sulfuris* ($\Delta\delta^{13}C$ = 0.3‰ and $\Delta\delta^{15}N$ = 3.8‰) (Fig.4b). The third polynoid *L. riftense* was much depleted in $\delta^{13}C$ (-15.5‰) could possibly be due to the POM contribution to its diet, not dissimilar to the polynoid *Lepidonotopodium* sp. sampled by Limén et al. (2007) (Fig.4b). Levesque et al. (2006) observed this latter species feeding directly on POM. It is possible that in our study in the long-term experiments in the siboglinid habitat (Table 3), the polychaete *L. riftense* may have fed directly on POM (Fig.4b). Indeed, the $\delta^{15}N$ was very light for this polychaete species, which is assumed to be a predator, and $\delta^{13}C$ was depleted compared to those of the other taxa (Fig.4a, b). Initially in Table 2, this polychaete was seen as a predator only, but in our study after one month this worm switches its diet to detritivore (Table 3).

4.4 Some insight on *Alvinella Pompejana*'s diet

According to the literature (references herein), the trophic fractionation (Δ) of both $\delta^{13}C$ and $\delta^{15}N$ between prey and predator is usually 0-1‰ and 3-4‰ respectively. Mc Cutchan et al. (2003) demonstrated that Δ of $\delta^{15}N$ can be around 2‰ between primary consumer and primary producer, which is closer to our calculations for the 2-week and 1-month experiments between the alvinellid

polychaete and its episymbionts. This indicates that episymbiotic communities could have been used as a food source by *Alvinella pompejana* during the 2-week and the one-month experiments. Pioneer authors (Desbruyères et al., 1983; Gaill et al., 1987; Desbruyères et al., 1998) working on epibiosis of *A. pompejana* suggested that these bacteria could be used as a food source by the alvinellid polychaetes. Analyses of lipid biomarkers were undertaken on both *A. pompejana* and its episymbionts (Phleger et al., 2005), but authors were unable to confirm the hypothesis that the worm was actually feeding on its episymbionts. The only conclusion of their analyses was that, thanks to the lipids, fatty acid and sterol profiles, there was strong evidence for bacterial dietary input for *A. pompejana* suggesting that they were bacterivores, which we also infer in this paper from the TP calculation (Table 3). In a recent paper (Grzymski et al., 2008), a specimen of *A. pompejana* was shown grazing on the back of another conspecific. Likewise, *Shinkaia crosnieri*, a vent galatheid crab, was observed grazing on its epibiotic bacteria in an aquarium (Miyake et al., 2007). It was hypothesized that this vent crustacean may harvest its episymbionts to feed, but that the crab also feeds on free living bacteria (Goffredi et al., 2008). Again, the limpet *Lepetodrilus fucensis* hosts filamentous episymbionts on its gill lamellae that may be ingested directly by the gill epithelium (Bates, 2007). Based on the stable isotopic values in the 2-week and 1-month experiments in our study, *A. pompejana* may have used its episymbionts or those from a conspecific as a food source during the TRAC experiments.

4.5 Consequences on the food web structure at a vent

Overall, food web structures (Fig.5) established within TRACs deployed both in the alvinellid and the siboglinid habitats appear to be similar to models described previously by Bergquist et al. (2007) from vent community issued from Juan de Fuca ridge and recently reviewed by Govenar (2012) from vent communities issued from both Juan de Fuca Ridge and East Pacific Rise. In general we have 3 to 4 trophic levels with multiple food sources for each consumer as seen

517 in Bergquist et al. (2007) and Govenar (2012) (Fig.5). Primary producers may be divided into three
518 groups, (1) free-living bacteria colonizing TRAC, mostly *Epsilonproteobacteria*, (2) episymbiotic
519 bacteria attached to invertebrates, (3) Particulate Organic Matter resulting from a mixture of detritus
520 from decaying bodies, mucus, microbial cells and photosynthetically-derived surface material and
521 associated bacteria. Primary consumers may be divided into two groups, (1) bacterivores feeding on
522 free-living bacteria within TRACs or on episymbiotic bacteria, (2) detritivores or scavengers feeding
523 on detritus issued from decomposition of free-living bacteria, episymbionts and invertebrates and
524 photosynthetically-derived surface material and associated bacteria. Secondary consumers are
525 predators feeding on primary consumers either bacterivores or detritivores. Spatial differences may
526 be observed in food web structures within TRACs deployed in alvinellids habitat dominated by
527 primary consumers (bacterivores and detritivores) compared to those deployed in siboglinid habitats,
528 where a higher number of predators (secondary consumers) seems to occur (Fig.4a, b; Table 3).
529 Temporal differences in food web structures within TRACs may be more highlighted in our study
530 both in the alvinellid and siboglinid habitats with an increase of detritivores compared to
531 bacterivores over time (Fig.4a, b; Table 3).

532 Within our study, trophic specialists (most of the species) that have narrow $\delta^{13}C$ values seems
533 to coexist with trophic generalists (dirivultid copepods and limpet) that have larger $\delta^{13}C$ values
534 (Table 4) confirming the hypothesis of resource partitioning at vent habitat (Bergquist et al., 2007).
535 However, De Busserolles et al. (2009) argued this hypothesis, indicating that the variance of $\delta^{13}C$
536 was more linked to the variations of environmental conditions and the breadth of the trophic niche,
537 concluding it was not the best tool to assess feeding strategies at vent. Using our data, we can agree
538 with these last authors as the two polynoids *Lepidonotopodium riftense* and *Branchinotogluma*
539 *sandersi* considered to be predators (secondary consumers) seen in Bergquist et al. 2007 (Table 2)
540 were seen as detritivores/scavengers (primary consumers) in this study for some of the colonization
541 experiments (Table 3). These two species have switched their diet evidence from a decrease or an

increase in the $\delta^{15}N$ values (and trophic levels) however the $\delta^{13}C$ ratios are narrow and not variable leading to specialist feeding strategies in theory, but in fact are more related to generalist strategies.

Plasticity in trophic relationships at vents seems to be common in secondary consumers; it does not only occur in Annelid polychaetes like this study, but was seen in Mollusk gastropods and in Arthropods crustacean in other studies at vents (Govenar, 2012). This switch in diet occurred also within our study in the primary consumer such as *Alvinella pompejana*, the gastropod *Lepetodrilus elevatus* and the dirivultid copepods. Paralvinellid polychaetes were seen at the Juan de Fuca Ridge to switch its bacterial diet to detritical diet while the fluid flux was diminishing (Levesque et al., 2003; 2005). This strategy to switch its diet may be an adaptation for taxa living at hydrothermal vents where hydrothermal fluids are unstable (Luther et al., 2001; Le Bris et al., 2006; this study), altering the availability of resources for chemosynthetic primary producers. This study may help to increase the knowledge of the complexity of the food web at hydrothermal vents and better understanding communities' resilience following environmental changes.

5 CONCLUSION

To conclude we demonstrated by using colonization experiments that some spatial heterogeneity in the $\delta^{13}C$ of consumers may be observed on a vent edifice at the meter scale, arising from the partitioning of food resources. Our study suggested that within a short period (one month), both $\delta^{13}C$ and $\delta^{15}N$ values of some primary and secondary consumers such as *Lepidonotopodium riftense*, *Branchinotogluma sandersi*, *Alvinella pompejana*, *Lepetodrilus elevatus* and dirivultid copepods varied greatly indicating first of all, a fast tissue turnover and secondly, a switch in feeding strategy or food sources consumption for some invertebrates taxa at this hydrothermal vent. Finally, through the analysis of stable isotopes ratios of alvinellid polychaetes and their episymbionts that we recovered in TRACs, we provided evidence that these attached bacteria may have formed part of the diet of these worms during these *in situ* experiments.

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841 **Table 1.** Details of TRACs (Titanium Ring for Alvinellid Colonization) deployment and recovery at the EPR 9°50'N in 2006.
842 Mean temperature data with standard deviation (± SD) were calculated according to the *in situ* temperature recording by
843 MICREL autonomous probes attached to TRACs.

TRAC ID	Localisation on vent sulfide edifice	Date of deployment (dive number)	Date of recovery (dive number)	Temperature range (°C)	Mean temperature (°C)	Bacterial mats present during recovery *(video in situ* observation)
4ALVT	*Alvinella* habitat	4 Nov (4263)	8 Nov (4267)	2.1-21.7*	8.6 ± 2.7 *	Yes
4TEVT	*Tevnia* habitat	4 Nov (4263)	8 Nov (4267)	2.4-7.9	5.2 ± 0.9	Yes (small patch)
4BAST	Bare basalt	4 Nov (4263)	8 Nov (4267)	1.9-4.8	2.4 ± 0.4	No
13ALVT	*Alvinella* habitat	17 Dec (4289)	30 Dec (4292)	Probe 1: 66.8->175.7	n.c.	Yes (everywhere)
				Probe 2: 10.6->243.1	58.3 ± 26.3 **	
13TEVT	*Tevnia* habitat	17 Dec (4289)	30 Dec (4292)	2.3-29	10.5 ± 4.9	Yes (few patches)
13BAST	Bare basalt	17 Dec (4289)	30 Dec (4292)	1.9-2.5	2.1 ± 0.1	No
33ALVB	*Alvinella* habitat	10 Nov (4269)	13 Dec (4285)	Probe 1: 1.9-14.8	6.3 ± 1.9	Yes (everywhere)
				Probe 2: 1.9-18.7	8.7 ± 2.4	
29TEVT	*Tevnia* habitat	14 Nov (4273)	13 Dec (4285)	1.9-31	5.3 ± 3.2	Yes (few patches)
29BAST	Bare basalt	14 Nov (4273)	13 Dec (4285)	1.9-2.5	2.0 ± 0.1	no

844 * Mean temperature and range are given for the 3 last days of the experiment, after the TRAC fell a few centimetres away from its original location.
845 ** Mean temperature calculated from measurements obtained in the first week, before temperature became consistently higher than the probe accuracy
846 limit (100°C).
847 n.c.: not calculated.
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855 **Table 2.** Density of the dominant taxa in dm^3 collected within the nine Titanium Ring for Alvinellid Colonization devices (TRACs) deployed

856 between November and December 2006 at the East Pacific Rise. TRACs are detailed in Table 1. Trophic guilds are after Bergquist et al. (2007):

857 B = bacterivore; S = scavenger/detritivore; P = predator; d = surface deposit-feeder or grazer, s = suspension feeder.

Taxonomic group	Trophic guild	Density of specimens per TRAC in dm^3								
		4ALVT	4TEVT	4BAST	13ALVT	13TEVT	13BAST	33ALVB	29TEVT	29BAST
Alvinella pompejana	B (d,s)	14	-	-	20	-	-	7	-	-
Branchinotogluma sandersi	P	-	1	-	-	1	-	-	1	-
Lepidonotopodium riftense	P	-	2	-	-	1	1	-	4	-
Branchinotogluma hessleri	P	-	-	-	-	2	-	2	-	-
Lepetodrilus elevatus	B (d,s)	-	143	18	4	1094	1	62	688	4
Dirivultid copepods	B (d)	565	113	47	327	86	9	-	-	-
Ventiella sulfuris	B	-	1	6	160	223	-	183	125	4
Total density per TRAC		581	286	71	517	1429	71	257	834	29

858 **Table 3.** Trophic position (TP) calculated following Post (2002) by using $\delta^{15}N$ of episymbionts as trophic baseline and trophic fractionation of
859 1.4‰ (invertebrate's diet) and 3.3‰ (microbial diet) (Bergquist et al., 2007). Species are those used for stable isotopes recovered from Titanium
860 Ring for Alvinellid Colonization devices (TRACs) deployed in both *Alvinella pompejana* and *Tevnia jerichonana* habitats. TRACs are detailed
861 in Table 1. Feeding guilds according to this study are in brackets and as follow: B for bacterivore, D for detritivore and P for predator.

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Taxonomic group	4ALVT	13ALVT	33ALVB	4TEVT	13TEVT	29TEVT
Trophic baseline						
Episymbionts (prokariotes)	1.0	1.0	1.0	-	-	-
Microbial diets and detritus						
Alvinella pompejana	1.4 (B)	1.4 (B)	1.6 (B)	-	-	-
Dirivultid copepods	1.7 (B)	1.6 (B)	-	0.8 (B)	1.6 (B)	-
Lepetodrilus elevatus	-	-	2.1 (D)	2.1 (D)	1.9 (B)	2.0 (D)
Ventiella sulfuris	-	2.0 (D)	2.1 (D)	-	2.0 (D)	2.1 (D)
Invertebrate diets						
Branchinotogluma sandersi	-	-	-	2.9 (D)	4.1 (P)	6.2 (P)
Lepidonotopodium riftense	-	-	-	5.2 (P)	3.9 (P)	2.2 (D)
Branchinotogluma hessleri	-	-	5.9 (P)	-	6.0 (P)	-

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870 **Table 4.** Summary of ranges of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios of similar invertebrates issued from the literature and
871 those targeted in this present study. EPR: East Pacific Rise. JdF: Juan de Fuca. HCl means that samples were acidifed. In this study, values of
872 $\delta^{13}C$ and $\delta^{15}N$ are the average and standard deviation (± SD) of the values measured for a given taxa recovered for any of the 6 colonization
873 experiments.

Sources	Species	Location	Fixation	HCl	Storage	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Desbruyères et al.,1983	*Alvinella pompejana*	EPR 21°N	Formaldehyde	yes	-	-11.2	6.0-6.8
Van Dover and Fry, 1989	*Alvinella pompejana*	EPR 21°N	Freezing	yes	-	-11.7	4.7
This study	*Alvinella pompejana*	EPR 9°50'N	Formaldehyde	no	Ethanol	-11.4 ± 0.8	6.5 ± 0.6
Van Dover and Fry, 1989	*Lepetodrilus sp.*	EPR 21°N	Freezing	yes	-	-12	9.2
Colaço et al., 2002	*Lepetodrilus sp.*	Menez Gwen	Freezing	yes	-	-20.1	2.49 ± 1.13
This study	*Lepetodrilus elevatus*	EPR 9°50'N	Formaldehyde	no	Ethanol	-13.3 ± 1.4	8.4 ± 0.2
Van Dover and Fry, 1989	*Lepidonotopodium riftense*	EPR 21°N	Freezing	yes	-	-11.6	8.1
This study	*Lepidonotopodium riftense*	EPR 9°50'N	Formaldehyde	no	Ethanol	-15.5 ± 0.3	8.9 ± 2.0
Bergquist et al., 2007	*Branchinotogluma hessleri*	JdF	Formaldehyde	yes	Ethanol	-15.8/-15.3	4.7/6.1
This study	*Branchinotogluma hessleri*	EPR 9°50'N	Formaldehyde	no	Ethanol	-12.8 ± 0.3	12.0 ± 0.2
Bergquist et al., 2007	*Branchinotogluma sandersi*	JdF	Formaldehyde	yes	Ethanol	-20.1/-16.1	4.4/5.8
This study	*Branchinotogluma sandersi*	EPR: 9°50'N	Formaldehyde	no	Ethanol	-11.5 ± 0.9	9.8 ± 2.4
Colaço et al., 2002	Amphipods	Menez Gwen	Freezing	yes	-	-21.6	1.8 ± 0.62
Fisher et al., 1994	*Ventiella sulfuris*	Galapagos	Freezing	yes	-	-24.1	2.3
This study	*Ventiella sulfuris*	EPR 9°50'N	Formaldehyde	no	Ethanol	-13.3 ± 0.3	8.5 ± 0.1
Limén et al., 2007	*Aphotopontius forcipatus*	JdF	Freezing	yes	-	-22.2/-13.5	4.7
Limén et al., 2008	*Stygiopontius quadrispinosus*	Northeast			-	-15.9/-13.2	-0.2/4.7
	Benthoxinus scupilifer	Pacific	Freezing	yes	-	-14.8/-13.1	-0.3/1.2
This study	Dirivultid copepods	EPR 9°50'N	Formaldehyde	no	Ethanol	-12.5 ± 1.4	6.3 ± 1.5

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875 Figure captions

876 **Fig. 1.** Map of location of Titanium Ring for Alvinellid Colonization (TRAC) experiments. Right:
877 East Pacific Rise showing Mexico City (*); left: detailed map of the 9º50'N segment.

878

879 **Fig. 2.** Deployment location of Titanium Ring for Alvinellid Colonization devices (TRACs) on the
880 TICA site. (A) TRAC (red arrow) deployed on *Alvinella pompejana* habitat (green asterisk). (B)
881 TRAC (red arrow) deployed on *Tevnia jerichonana* habitat (blue circle). (C) TRAC (red arrow)
882 deployed directly on fresh bare basalt. The dark line represents the outline of the sulfide edifice
883 viewed laterally. The scale bars represent 1 metre.

884

885 **Fig. 3.** Semi-continuous temperature measurements during *in situ* deployment of TRACs (Titanium
886 Ring for Alvinellid Colonization). A) Deployments (depl.) on fresh bare basalt. B) Deployments
887 (depl.) at the base of *Tevnia jerichononana* tubeworms. C) Deployments (depl.) on alvinellids, where
888 for the 13-day and 33-day deployments, two MICREL probes where attached to TRACs giving two
889 temperature records in black and grey colours. The arrow in the 4-day deployment indicates when
890 the TRAC moved from its original position. Please note that temperatures higher than 100°C (probe
891 accuracy limit) in the 13-day deployment should be taken with caution.

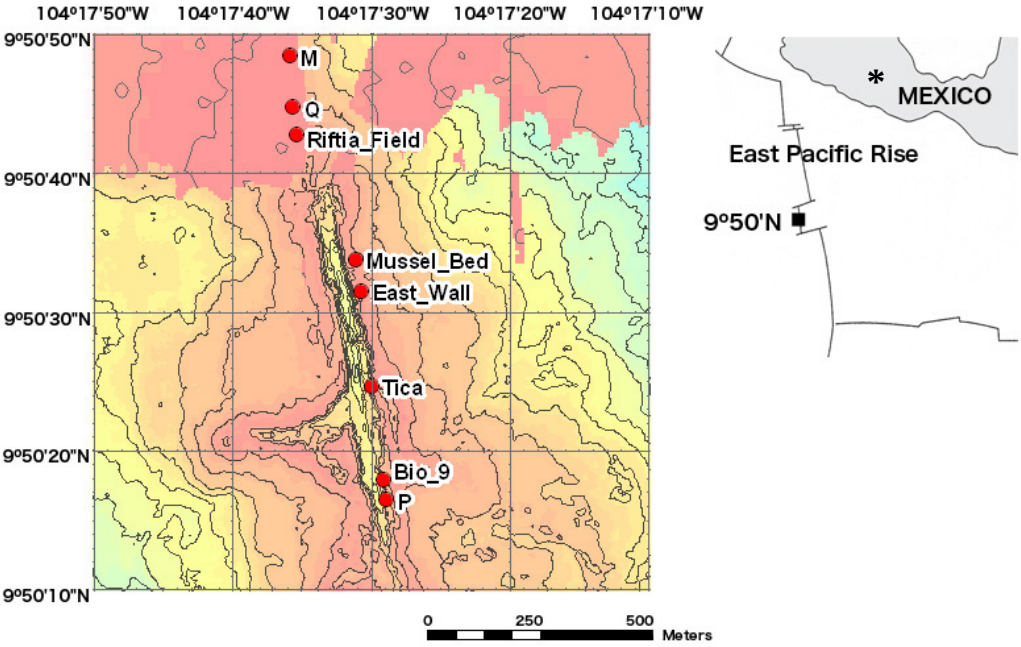
892

893 **Fig. 4.** $\delta^{13}C$ and $\delta^{15}N$ values of dominant taxa in the Titanium Ring for Alvinellid Colonization
894 (TRACs) (A) in the *Alvinella pompejana* habitat and (B) in the *Tevnia jerichonana* habitat. Numbers
895 within symbols represent the number of days *in situ* of TRAC deployments from which the taxa was
896 recovered. Dashed grey boxes represent the two other potential primary producers where $\delta^{13}C$ and
897 $\delta^{15}N$ for particle organic matter (POM) was taken from the diffuse flow of a vent at Juan de Fuca
898 Ridge (Limén et al., 2007), in (A) originating from the communities of *Paralvinella sp.* that live
899 close to the vent openings: $\delta^{13}C$ = -16.7 to -17.2‰ and $\delta^{15}N$ = 4.6 to 6.6 ‰, in (B) originating from

an intermediate habitat of a sulfide edifice: $\delta^{13}C$ = -18.3 to -19.3‰ and $\delta^{15}N$ = 4.6 to 7.4 ‰. In (A) and (B) Chemoautotrophic Bacteria (Chemo. Bact.) are from Campbell et al. (2003) where δ13C = -12‰ and -8‰ and, $\delta^{15}N$ = 0-4 ‰. Error bars are standard deviations of the mean; *n* = 5 for *A. pompejana*; pools of 3 specimens for *Lepidonotopodium riftense* and for *Branchinotogluma hessleri*; pools of 2 specimens for *Branchinotogluma sandersi*, pools of 20 specimens for *Lepetodrilus elevatus* and for *Ventiella sulfuris* and pools of 100 specimens for the dirivultid copepods.

Fig. 5. Synthesized food web for community recovered within Titanium Ring for Alvinellid Colonization (TRAC) at the TICA site in November and December 2006 based on Bergquist et al. (2007) and Govenar (2012) but modified according to this study. Trophic guilds are those calculated from Table 3. Arrows indicate direct consumption.

Figure 1


104°17'50"W
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9°50'20"N
9°50'10"N
M
Q
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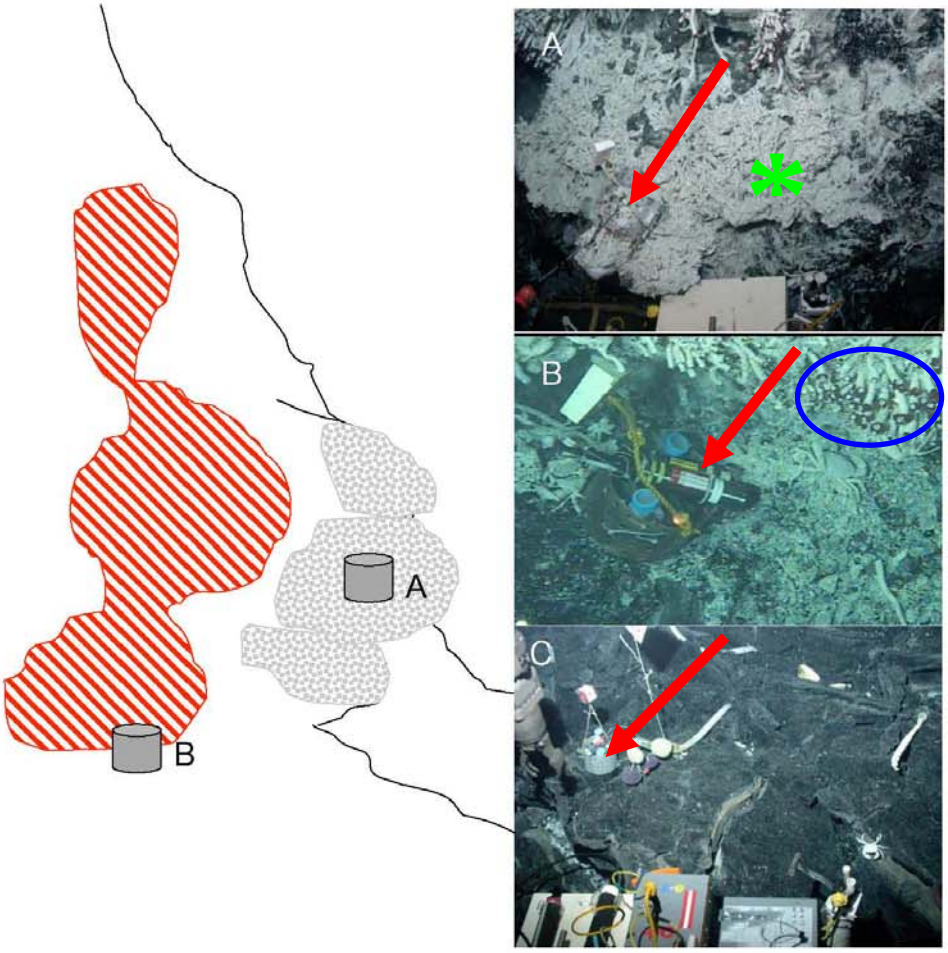
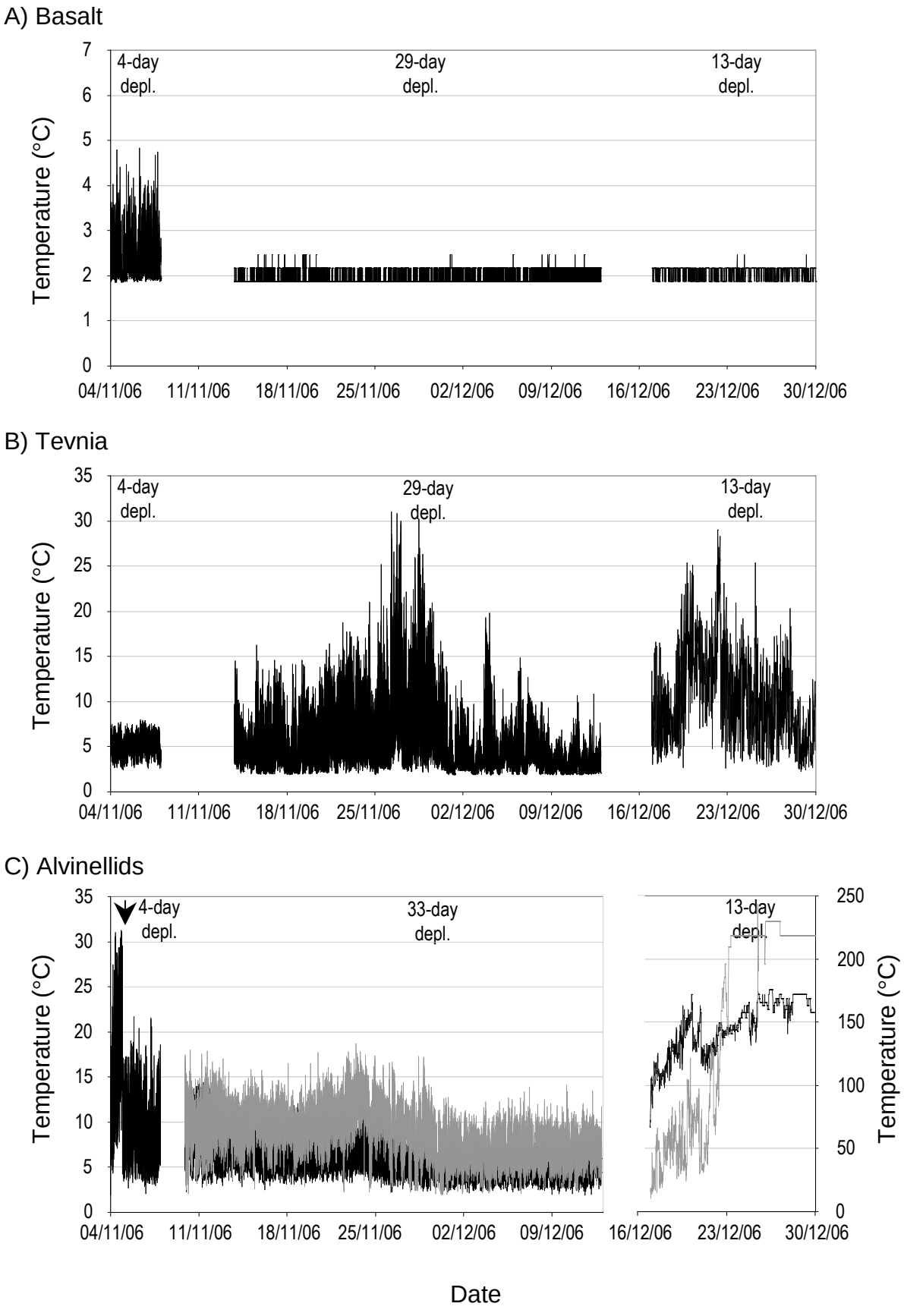
MEXICO
East Pacific Rise
9°50'N

Figure 2



A. *Alvinella pompejana* habitat (4ALVT, 13ALVT)
B. *Tevnia jerichonana* habitat (4TEVT, 13TEVT, 29TEVT)
C. Bare basalt habitat (4BAST, 13BAST, 29BAST)

Figure 3


A) Basalt
4-day depl.
29-day depl.
13-day depl.
Temperature (°C)
0
1
2
3
4
5
6
7
04/11/06
11/11/06
18/11/06
25/11/06
02/12/06
09/12/06
16/12/06
23/12/06
30/12/06
B) Tevnia
4-day depl.
29-day depl.
13-day depl.
Temperature (°C)
0
5
10
15
20
25
30
35
04/11/06
11/11/06
18/11/06
25/11/06
02/12/06
09/12/06
16/12/06
23/12/06
30/12/06
C) Alvinellids
4-day depl.
33-day depl.
13-day depl.
Temperature (°C)
0
5
10
15
20
25
30
35
0
50
100
150
200
250
Temperature (°C)
04/11/06
11/11/06
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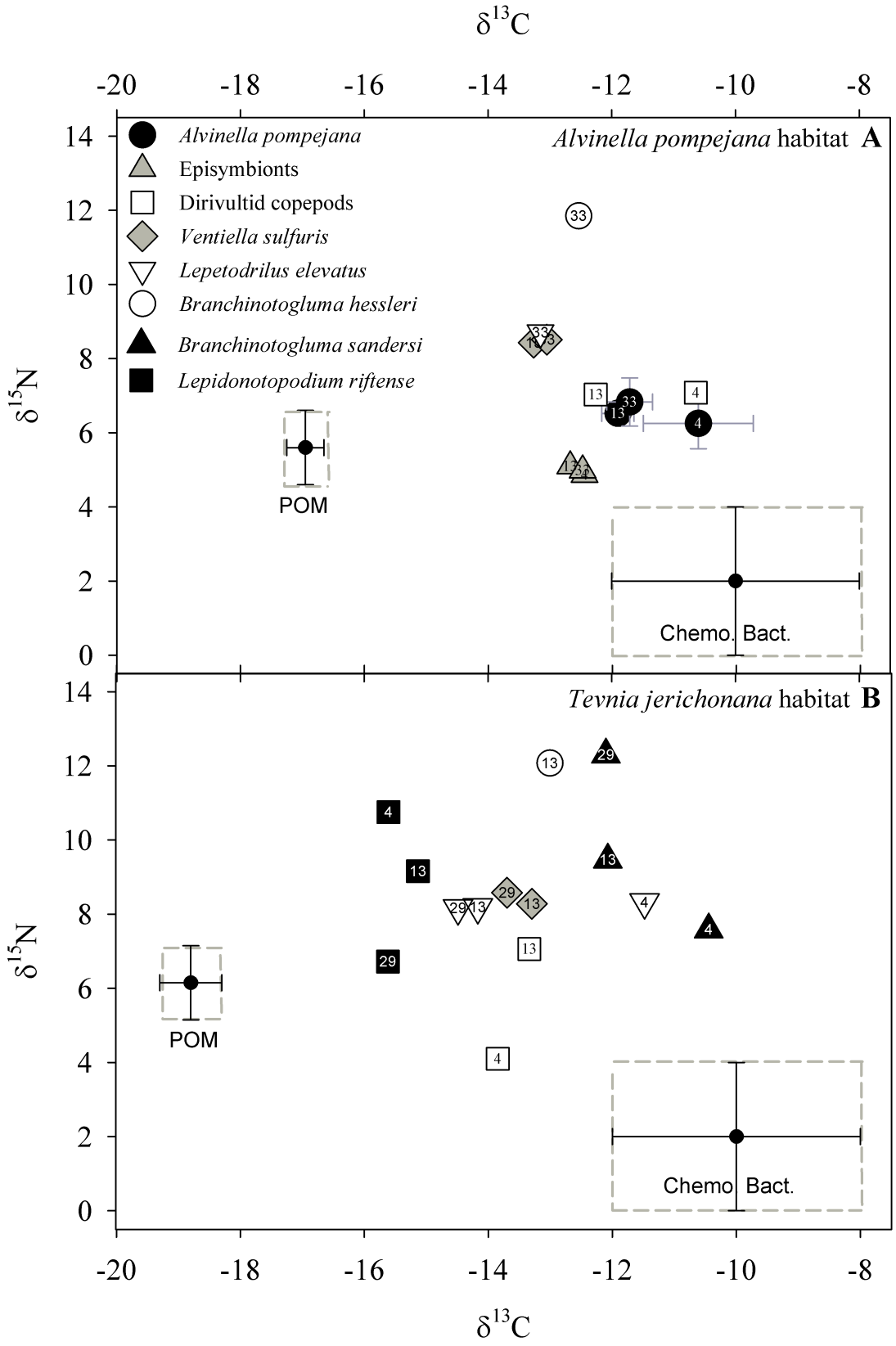
25/11/06
02/12/06
09/12/06
16/12/06
23/12/06
30/12/06
Date

Figure 4


δ13C
-20
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δ15N
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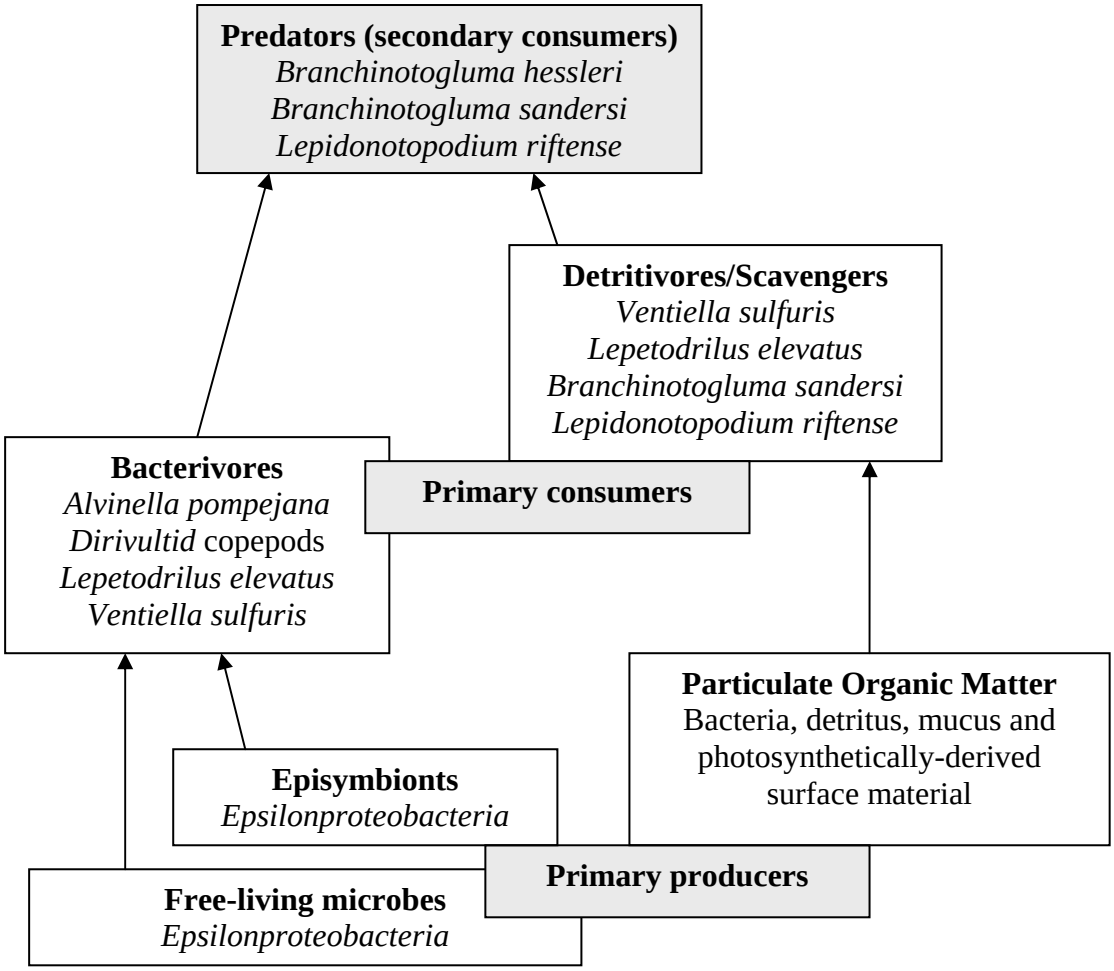
Alvinella pompejana
Episymbionts
Dirivultid copepods
Ventiella sulfuris
Lepetodrilus elevatus
Branchinotogluma hessleri
Branchinotogluma sandersi
Lepidonotopodium riftense
Alvinella pompejana habitat A
POM
Chemo. Bact.
Tevnia jerichonana habitat B
POM
Chemo. Bact.

Figure 5


Predators (secondary consumers)
Branchinotogluma hessleri
Branchinotogluma sandersi
Lepidonotopodium riftense
Detritivores/Scavengers
Ventiella sulfuris
Lepetodrilus elevatus
Branchinotogluma sandersi
Lepidonotopodium riftense
Bacterivores
Alvinella pompejana
Dirivultid copepods
Lepetodrilus elevatus
Ventiella sulfuris
Primary consumers
Particulate Organic Matter
Bacteria, detritus, mucus and photosynthetically-derived surface material
Episymbionts
Epsilonproteobacteria
Primary producers
Free-living microbes
Epsilonproteobacteria