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How are microbial and detrital sources partitioned among and within gastropods species at East Pacific Rise hydrothermal vents?

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Abstract

For the last few decades, trophic ecology has usually been investigated by using stable isotopes. However, the isotopic signatures of potential food sources in hydrothermal vent ecosystems are often unknown and so their relative contribution to the consumers' diet, as well as resource partitioning, are then difficult to estimate. Here, we used a recent Bayesian mixing model (stable isotope analysis in R, SIAR) based on $\delta^{13}C$ and $\delta^{15}N$ to estimate the contribution of multiple food sources to the diet of eight vent gastropods that can reach high densities at hydrothermal vents (*Lepetodrilus elevatus*, *Lepetodrilus pustulosus*, *Lepetodrilus ovalis*, *Eulepetopsis vitrea*, *Cyathermia naticoides*, *Peltospira delicata*, *Peltospira operculata* and *Rhynchopelta concentrica*). These species, known as primary consumers (mostly bacterivores and detritivores), were sampled on the South-East Pacific Rise at 17°25′ S and the North-East Pacific Rise at 9°50′ N and 12°50′ N. Several potential food sources were sampled according to the gastropod habitat on the chimney wall, or mussel beds (proxies of *Gammaproteobacteria* form I RubisCO, *Gammaproteobacteria* form II RubisCO and *Epsilonproteobacteria*, biofilms of siboglinid and alvinellid tubes, biofilms of mussel shells and particulate organic matter). Some of these microbial and detrital sources were confirmed as present in the gut content of some small specimens by transmission electron microscopy. Distinct stable isotopic signatures of the potential food sources allowed calculation of their relative contributions to primary consumers' diets. This revealed that gastropod species living on siboglinid or bathymodiolin habitats are usually generalists, feeding on various pools of microbial or detrital origins. For a particular habitat, sympatric gastropod species partition the food sources, thus avoiding being in competition. Only for the alvinellid habitat *Peltospira* spp. appeared to be more specialists as the choice of food sources is more reduced.

Introduction

Hydrothermal food webs are mainly fueled by chemosynthetic primary production, which results from the oxidation of inorganic, reduced compounds, creating oases of endemic vent fauna in the otherwise desert-like deep sea (Van Dover 2000). At hydrothermal vents, diverse chemolithoautotrophic bacteria (*Alphaproteobacteria*,

Gammaproteobacteria, *Epsilonproteobacteria*) and Archaea employ four different metabolic pathways to fix inorganic carbon: (i) the Calvin–Benson–Bassham (CBB) cycle; (ii) the reductive tricarboxylic acid (rTCA) cycle; (iii) the 3-hydroxypropionate (3-HP) cycle; and (iv) the reductive acetyl coenzyme A (acetyl-CoA) cycle (Campbell *et al.* 2006; Nakagawa & Takai 2008). The energy comes from the oxidation of hydrogen and sulfur compounds, coupled with the reduction of oxygen, nitrate and sulfur compounds (Nakagawa & Takai 2008). Methane oxidation is an alternative, which acts as both a carbon and energy source (Childress *et al.* 1986; Fujiwara *et al.* 2000). These prokaryotes either occur free-living on hard surfaces (tubes, shells, *etc.*), in the water column or as symbionts of metazoan species, being either episymbionts attached to the external surface (*e.g. Alvinella pompejana* or *Rimicaris exoculata*; Campbell *et al.* 2003; Zbinden *et al.* 2004) or endosymbionts located within organs such as the trophosome in siboglinid polychaetes (Cavanaugh *et al.* 1981; Felbeck 1981) or within gill epithelial cells in mytilid bivalves (Duperron 2010). These autotrophic prokaryotes are considered to be at the base of the vent food web (Govenar 2012). However, an alternative source for primary consumers is particulate organic matter (POM; Limén *et al.* 2007). POM is composed of a mixture of endogenous sources from the hydrothermal vent (detritus from decaying bodies, mucus and microbial cells) and exogenous sources from the surrounding sea, such as small fractions of photosynthetically derived surface material and associated bacteria (according to Levesque *et al.* 2005). POM composition varies with proximity to fluid-emission, becoming more detrital in nature and depleted in ^{13}C with increasing distance (Levesque *et al.* 2005; Limén *et al.* 2007). All of these food sources provide a variety of potential primary sources at the base of the vent food web.

The hydrothermal ecosystem is an unstable environment where sulfide concentration and temperature fluctuate at different spatial and temporal scales (Le Bris *et al.* 2006). At its highest naturally occurring concentrations, sulfide is also deadly to most animals. Vent endemic organisms have evolved physiologic and behavioral adaptations to cope with these harsh living conditions, such as using symbiosis for detoxification (Pruski & Fiala-Médioni 2003), living in aggregates (such as the shrimp *Rimicaris exoculata*, Schmidt *et al.* 2008) and living within protective tubes (*e.g.* alvinellid polychaetes, Le Bris *et al.* 2005; Ravaux *et al.* 2013). Sulfide and thermal tolerances differ among species, leading to ecological-niche partitioning and zonation of species on vent edifices (Sarrazin & Juniper 1999; Bates *et al.* 2005; Cuvelier *et al.* 2011; Marsh *et al.* 2012). This zonation is also partly controlled by biotic factors, principally competition and predation (Micheli *et al.* 2002).

Gastropods within diverse genera occur in different zones on vent edifices, reaching high densities and diversity (Govenar *et al.* 2005; Mills *et al.* 2007; Matabos *et al.* 2008a; Matabos *et al.* 2011; Pradillon *et al.* 2009; Gaudron *et al.* 2012). Being mostly grazers, but also filter-feeders, they are among the main primary consumers in the vent ecosystem (Warén & Bouchet 1993; Bates 2007a). A few of them also host symbionts (Bates 2007b; Beinart *et al.* 2012). Different species may compete for resources when living in the same zone if their trophic niches overlap, for example by feeding on the same food source (*i.e.* the same type of bacteria) as seen in paralvinnelid polychaetes from Juan de Fuca Ridge (Northeast Pacific) at high hydrothermal flux (Levesque *et al.* 2003). Alternatively, species may partition the resources within a targeted zone to avoid being in competition, as seen in paralvinellid polychaetes at low hydrothermal flux feeding either on POM or on bacteria depending on the species (Levesque *et al.* 2003). In order to address whether competition or food partitioning occurs among gastropods, inter-specific diet comparisons are needed spatially.

A large number of trophic ecology studies at vents and in other ecosystems (see Boecklen et al 2011 for review) have used stable carbon and nitrogen isotopes signatures to address nutrition. What has been missing so far in vent trophic ecology studies is determination of the stable isotopes signatures of the potential food sources (*i.e.* free *Alphaproteobacteria*, *Gammaproteobacteria*, *etc.*). This contrasts with other marine systems, such as coastal marine ecosystems (Marín Leal *et al.* 2008) in which stable isotopes signatures of primary producers are easily sampled, measured and known (*i.e.* riverine POM, POM, microphytobenthos, macroalgae, *etc.*). With this information on hand one may use a mixing model to estimate the relative contribution of a primary producer to a primary consumer, such as in the Channel Sea in North-Eastern Atlantic with several filter-feeders (bivalves and polychaetes) where four potential food sources contributed to the diet of these primary consumers (Lefebvre et al 2009). For vent systems, to our knowledge only two studies (Limén *et al.* 2007; Sweetman *et al.* 2013) have used a multi-sources mixing model (*i.e.* IsoSource, Phillips & Gregg 2003) to assess the relative contribution of a primary source to the diet of a vent primary consumer.

In this study we analysed $\delta^{13}C$ and $\delta^{15}N$, both in species of gastropods sampled at different sites along the East Pacific Rise (EPR), and in potential microbial and detrital sources. These stable isotope signatures were implemented within a Bayesian mixing model (stable isotope analysis in R, SIAR; Parnell *et al.* 2010), currently used in trophic studies to decipher food partitioning. It estimates the relative contributions of multiple resources to the diet of a primary consumer, although it has not

yet been applied to hydrothermal trophic ecology. SIAR allowed the use of more sources than isotopes, while also including variability in the stable isotope values for the consumers, the sources and any trophic enrichment factors (TEFs; when these were available). The aims of our study were (i) to test our ability to isotopically differentiate a great number of microbial sources with distinct metabolisms; (ii) to estimate the contribution of diverse microbial and detrital sources to the diet of the eight vent gastropods species using the Bayesian mixing model; and (iii) to identify whether gastropods were competing or partitioning food sources among themselves.

Material and Methods

Study area

All sampling sites were basalt hosted vent systems in the EPR. Three main areas were investigated: two north at EPR 9°50′ N (2500 m deep) and at EPR 12°50′ N (2630 m deep) in 2010, one north at EPR 9°50′ N in 2012 (Fig. 1A–C) and one south at EPR 17°25′ S (2585–2589 m deep) in 2004 (Fig. 1D).

In winter 2005–2006, a volcanic eruption occurred at EPR 9°50′ N (Fig. 1A and B). After this, animal communities, large aggregates of the siboglinid *Tevnia jerichonana* and alvinellid polychaete colonies, re-established themselves around new, vigorously active vents (Nees *et al.* 2009). In 2010, there were still isolated occurrences of *T. jerichonana* but many had been replaced by the siboglinid *Riftia pachyptila* (Fig. 1E), and by 2012 no *T. jerichonana* were observed. Close to vent openings within the alvinellid habitat dominated by *Alvinella pompejana* (Fig. 1F), some incidental extreme high temperatures (T °C) were sometimes recorded [*i.e.* up to 150 °C, recorded in 2006 by a MICREL autonomous probe by Gaudron *et al.* (2012)], whereas Contreira-Pereira *et al.* (2013) measured T °C up to 27 °C in 2010 and 2012 using a compact autonomous voltammetric sensor, as well as highly elevated concentrations of sulfide (hereafter [H_2S]) ranging between 100 and 450 μM. In 2010 and 2012 within the *R. pachyptila* siboglinid-dominated habitat (Fig. 1E), Contreira-Pereira *et al.* (2013) measured [H_2S] between 20 and 140 μM and T °C between 2.7 and 11 °C. Farther away on the basalt, proximally to diffuse fluid emission, were patches of *Bathymodiolus thermophilus* that defined the mussel habitat [Fig. 1G; *i.e.* 0–70 μM [H_2S] and T °C between 2.7 and 11 °C measured in 2010 and 2012 by Contreira-Pereira *et al.* (2013)]. All three habitats were inhabited by dominant megafauna foundation species, known to harbor symbionts (Govenar 2010).

At EPR 12°50′ N, the Genesis vent site (Fig. 1C) was very active. No chemical records were available for the years 2010 and 2012. However, Le Bris *et al.* (2003) described this vent site as low in iron, acidic in pH, enriched in CO_2 and exposed to high levels of sulfide. Faunal assemblages were similar to previous sites at EPR 9°50′ N with alvinellid patches around smokers, where temperature ranged from 3 to 30 °C and sulfide between 72 and 1520 μM (Le Bris *et al.* 2003). Within *R. pachyptila* clumps located on cliffs of chimney walls, the temperature varied between 2 and 8 °C and sulfide between 3 and 31 μM (Le Bris *et al.* 2003).

At EPR 17°25′ S (Fig. 1D), in 2004, vent sites were more senescent, characterized by large patches of mussels (*B. thermophilus*), clams (*Calyptogena manignifica*) and stalked cirripedes with very rare clumps of *R. pachyptila* and no visible colonies of *A. pompejana* (Jollivet *et al.* 2004; Sarrazin *et al.* 2006). Discrete temperature measurements performed within a mussel assemblage ranged between 2.0 and 8.7 °C with an average value of 3.8 ± 1.7 °C (Sarrazin *et al.* 2006). No chemical data were available for the year 2004.

Experimental design and sampling

All gastropods and their potential food sources (Table 1) were sampled by the submersible *Nautile* in 2004, 2010 and 2012 during three cruises: BIOSPEEDO'04, MESCAL 1 and MESCAL 2 (chief scientists: Didier Jollivet, François Lallier and Nadine Le Bris, respectively) on the EPR (Fig. 1A).

Gastropods

Eight different gastropods species were sampled. *Lepetodrilus elevatus* (Lepetodrilidae; Fig. 2A) is one of the most dominant gastropod species at EPR 9°50′ N (Mills *et al.* 2007), at EPR 12°50′ N (Matabos *et al.* 2008b; Matabos *et al.* 2011) and at EPR 17°25′ S (Matabos & Thiébaut 2010), living in 'warm' habitats preferentially on siboglinid habitat (Mills *et al.* 2007; Gaudron *et al.* 2012) but occasionally on alvinellid and mussel habitats (Mills *et al.* 2007; Pradillon *et al.* 2009; Gaudron *et al.* 2012). *Lepetodrilus pustulosus* (Lepetodrilidae; Fig. 2B), *Cyathermia naticoides* (Neomphalidae; Fig. 2C) and *Rhynchopelta concentrica* (Peltospiridae; Fig. 2D) are less abundant compared with *Lepetodrilus elevatus* but are also recovered preferentially along the EPR on siboglinid habitat but also occasionally on mussel beds (Mills *et al.* 2007). *Lepetodrilus ovalis* (Vestigastropoda, Lepetodrilidae; Fig. 2E) and *Eulepetopsis vitrea* (Vestigastropoda, Neolepetopsidae; Fig. 2F) belong to the 'cool' group according to Mills *et al.* (2007) living in a cold habitat, which was regarded as 'the suspension-feeder zone' at EPR 9°50′ N. The two neomphalids *Peltospira delicata* (Fig. 2G) and *Peltospira operculata* (Neomphalina, Peltospiridae; Fig. 2H) have

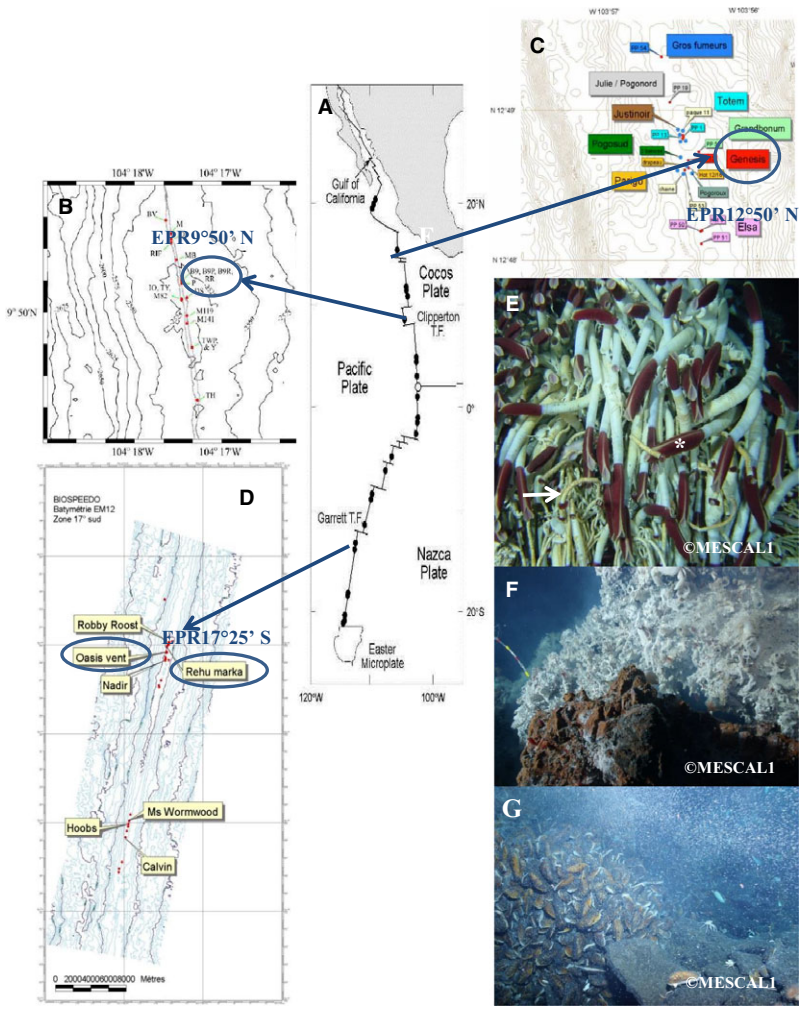

Fig. 1. Maps and photos of East Pacific Rise (EPR) hydrothermal vents in the Pacific Ocean. (A) General map of EPR plate; (B) details of hydrothermal vent sites at EPR 9°50′ N visited in 2010 and 2012 during the MESCAL 1 and 2 cruises; (C) details of hydrothermal vent sites at EPR 12°50′ N visited in 2010 during the MESCAL 1 cruise; (D) details of hydrothermal vent sites visited at EPR 17°25′ S in 2004 during the BIOSPEEDO '04 cruise; (E) photo taken *in situ* by the *Nautile* submersible of *Riftia pachyptila* (*)-dominated habitat in 2010 where *Tevnia jerichonana* is present (arrow); (F) photo taken *in situ* by the *Nautile* submersible of *Alvinella pompejana*-dominated habitat in 2010; (G) photo taken *in situ* by the *Nautile* submersible of *Bathymodiolus thermophilus*-dominated habitat. Photo credits: IFREMER.

commonly been reported on alvinellid habitat at both EPR 9°50′ N (Mills *et al.* 2007) and EPR 12°50′ N (Matabos *et al.* 2008a).

Identification of potential food sources

In *Alvinella pompejana* habitats (close to the vent openings), *Epsilonproteobacteria* dominate the free-living prokaryote community at EPR 9°50′ N (Campbell *et al.* 2006). Episymbionts covering the dorsal body of the polychaete *A. pompejana* are also dominated by *Epsilonproteobacteria* and may be regarded as a potential food source. Within siboglinid habitats, the tissues of tubeworms may be considered proxies for the sulfur-oxidizing bacteria (SOX) that they harbor within their trophosome and that can be also found as free-living forms on the chimney wall [such as assumed Limén *et al.* (2007) with *Ridgeia piscesae* at the Juan de Fuca Ridge]. Within mussel beds, *Bathymodiolus thermophilus* gills may be used as a proxy for the free-living chemolithoautotrophic bacteria (other SOX) that may also be present within the habitat. Several types of biofilms (heterotrophic/autotrophic bacteria and fungi) coating different hosts (mussel shells and tubes of siboglinid polychaetes) may also be used as potential food sources (Fretter 1988). *Riftia* tubes could also act as a potential food source as cut-off pieces of tubes have been also reported in the stomach of lepetodrilids living in siboglinid clumps (Fretter 1988). POM will vary in composition according to its proximity to the vent openings (Levesque *et al.* 2005). In 2004, POM was obtained by the collection of water samples using a Niskin bottle (8 l) at both sites (Oasis and Rehu Marka; Fig. 1D) about 1–2 m above the mussel beds and siboglinid clumps. In 2012, POM was taken from water samples recovered while sampling *A. pompejana* in alvinellid habitat. The biobox used was sterile before use and hermetically sealed during ascension to the surface to avoid environmental contamination. Similar to Gaudron *et al.* (2012), for both 2010 and 2012, the POM values used for the intermediate habitat (siboglinid) were from Limén *et al.* (2007) that was sampled at Juan de Fuca Ridge.

Table 1. Details of the gastropods and potential food sources used for stable isotope analysis in R (SIAR) sampled in the East and South-East Pacific Rise (EPR).

	biological material (gastropod species and biological sources for diet)	localization on EPR	type of habitat	date of sampling (dive number)	replicates (acidified)	observed by TEM
species collected in 2004 Biospeedo cruise	*Lepetodrilus elevatus*	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(10)	–
	Lepetodrilus pustulosus	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(10)	–
	Lepetodrilus ovalis	17°25′ S (site Rehu Marka)	17HB	05/01/2004 (dive 1590)	(10)	–
	Eulepetopsis vitrea	17°25′ S (site Rehu Marka)	17HB	05/01/2004 (dive 1590)	(10)	–
sources used with SIAR for species collected in 2004 (five sources)	(1) biofilm of *Riftia pachyptila*	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(1)*	–
	(2) Tubes of *Riftia pachyptila* and plume of *Riftia pachyptila*	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(2)	–
	(3) biofilm of *Bathymodiolus thermophilus*	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(1)*	–
	(4) *Bathymodiolus thermophilus* tissues	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(1)*	–
	(5) POM	17°25′ S (site Oasis)	17HBHR	05/01/2004 (dive 1590)	(2)	–
	POM	17°25′ S (site Rehu Marka)	17HB	05/01/2004 (dive 1590)	(1)	–
species collected in 2010 Mescal 1 cruise	*Lepetodrilus elevatus*	9°50′ N (site TICA)	9HR	05/14/2010 (dive 1734)	6	X
	Lepetodrilus elevatus	9°50′ N (site V vent)	9HB	05/13/2010 (dive 1733)	6	X
	Lepetodrilus elevatus	12°50′ N (site Genesis)	12HR	05/11/2010 (dive 1731)	4	–
	Cyathermia naticoides	9°50′ N (site Bio_9)	9HR	05/25/2010 (dive 1743)	4	X
	Cyathermia naticoides	12°50′ N (site Genesis)	12HR	05/11/2010 (dive 1731)	5	–
sources used with SIAR for species collected in 2010 (five sources)	(1) biofilm of *Riftia pachyptila*	9°50′ N (site Bio_9)	9HR	03/16/2012 (dive 1814)	1*	–
	(2) tubes of *Riftia pachyptila*	9°50′ N	9HR	2010	3 (3)	–
	(3) biofilm of *Bathymodiolus thermophilus*	9°50′ N (site Bio_9)	9HR	03/16/2012 (dive 1814)	1*	–
	(4) tissues of *Bathymodiolus thermophilus*	9°50′ N (site Bio_9)	9HB	03/16/2012 (dive 1814)	3	–
	(5) POM	From Limén & Levesque (2007)	9HB			
species collected in 2012 Mescal 2 cruise	*Peltospira delicata*	9°50′ N (site Bio_9)	9HA	03/20/2012 (dive 1818)	2	–
	Peltospira operculata	9°50′ N (site P vent)	9HA	03/23/2012 (dive 1821)	2	X
	Rhynchopelta concentrica	9°50′ N (site Bio_9)	9HBHR	03/16/2012 (dive 1814)	6	–
	Lepetodrilus pustulosus	9°50′ N	9HBHR	03/21/2012	3	–
sources used with SIAR for species collected in 2012 (three sources for species living on alvinellid habitat and five for the other species)	1) biofilm of *Alvinella pompejana*	9°50′ N (site Bio_9)	9HA	03/14/2012 (dive 1812)	2 (2)	–
	2) episymbiont of *Alvinella pompejana*	9°50′ N (site Bio_9)	9HA	03/14/2012 (dive 1812)	1*	–
	3) POM *Alvinella*	9°50′ N (site Bio_9)	9HA	03/16/2012 (dive 1814)	2 (2)	–
	1) *Oasisia alvinae*	9°50′ N	9HR	03/2012	3*	–
	2) biofilm of *Riftia pachyptila*	9°50′ N (site Bio_9)	9HR	03/16/2012 (dive 1814)	1*	–
	3) biofilm of *Bathymodiolus thermophilus*	9°50′ N (site Bio_9)	9HB	03/16/2012 (dive 1814)	1*	–
	4) tissues of *Bathymodiolus thermophilus*	9°50′ N (site Bio_9)	9HB	03/16/2012 (dive 1814)	3	–
	5) POM	From Limén *et al.* (2007)				

Note that some tissues were pooled (*) to get enough material for stable isotope analyses. Acidified samples were used for $\delta^{13}C$ analyses and non-acidified samples were used for $\delta^{15}N$ analyses in both 2010 and 2012. In 2004, all samples were acidified.

HB, *Bathymodiolus thermophilus*-dominated habitat (mussel habitat); HR, *Riftia pachyptila*-dominated habitat (siboglinid habitat); HA, *Alvinella pompejana*-dominated habitat (alvinellid habitat); HBHR, habitat with a mixture of *Riftia pachyptila* and *Bathymodiolus thermophilus*; POM, particulate organic matter; TEM, transmission electron microscopy.

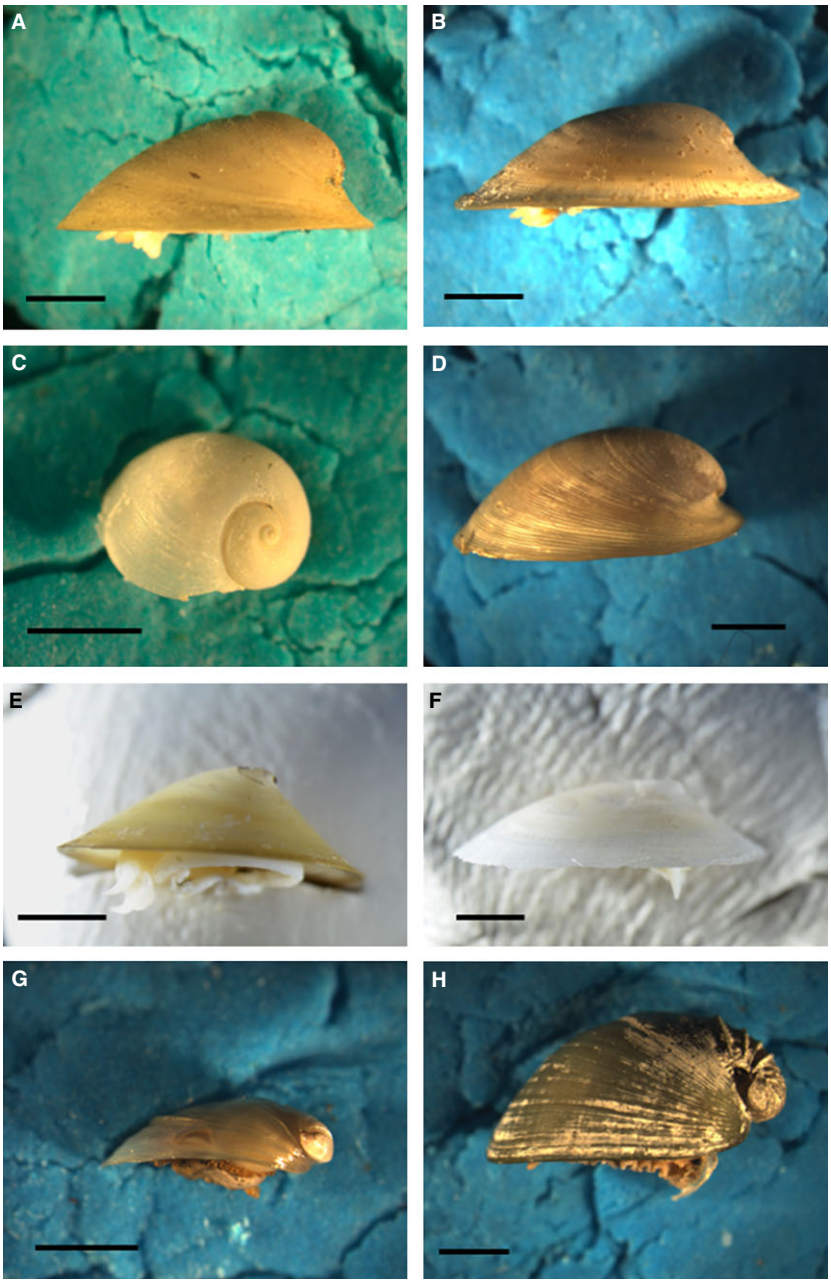

Fig. 2. The eight gastropod species sampled at East Pacific Rise (EPR) 9°50′ N for (A–D, G and H), EPR 12°50′ N for (A and C), and at EPR 17°25′ S for (A, B, E and F) among different habitats (alvinellid, siboglinid and mussel). (A) *Lepetodrilus elevatus*; (B) *Lepetodrilus pustulosus*; (C) *Cyathermia naticoides*; (D) *Rhynchopelta concentrica*; (E) *Lepetodrilus ovalis*; (F) *Eulepetopsis vitrea*; (G) *Peltospira delicata*; (H) *Peltospira operculata*. A and C were sampled in 2010; B, D, G and H were sampled in 2012; and E and F were sampled in 2004. Scale bars = 2 mm.

Sample treatments

Once on board, the fauna and potential food sources were sorted in a cold room with a dissecting microscope.

Primary consumers

Most gastropod species were frozen whole upon recovery for stable isotope analysis but for *Peltospira operculata*, *Lepetodrilus elevatus* and *Cyathermia naticoides*, the digestive tracts of a few specimens were dissected and then fixed in 2.5% glutaraldehyde for 4–16 h in filtered seawater, and then preserved in seawater-NaN_3 solution until used for observation by TEM. Tissues were then post-fixed in osmium, dehydrated in an increasing ethanol series (50, 70, 95 and 100°) and embedded in epoxy resin (48 h, 60 °C). Sections were cut using a Reichert–Jung ultramichrotome. Semi-thin (800 nm) sections were stained with toluidine blue and thin (50 nm) sections were mounted on copper grids, contrasted using uranyl acetate and observed using a HITACHI H-7100 transmission electron microscope operated at 80 kV. These TEM observations were used as a complement

to support the results provided by the stable isotope analyses.

Potential food sources

Biofilms were scraped from mussel shells (*Bathymodiolus thermophilus*) and polychaete tubes (*Alvinella pompejana* and *Riftia pachyptila*) in 2004 and 2012 using tweezers or a razor blade (Table 1). Episymbionts of *A. pompejana* were removed using tweezers in 2012 under a dissecting microscope (Table 1). The plume of *R. pachyptila* were dissected in 2004, and the trophosome of *Oasisia alvinae* in 2012 (Table 1). In 2004 and 2010, tubes of *R. pachyptila* were also sampled (Table 1). Mussel tissues were dissected in 2004 and 2012. POM in 2004 and 2012 was obtained after filtration of the sampling waters on pre-combusted Whatman glass-fiber filters.

Stable isotope analyses and mixing model

Frozen gastropods were dissected under a dissecting microscope to remove the shell. Specimen tissues and solid food sources were rinsed in distilled water and then dried (2 days, 60 °C). Some dried subsamples, such as the POM and biofilms collected from different metazoan species (Table 1), were treated with 0.1 N HCL (~3 h) to remove inorganic carbon and then rinsed with distilled water. This procedure was used because decarbonation has been reported to affect the $\delta^{15}N$ and total organic N values (Ryba & Burgess 2002). Both the dried decarbonated and carbonated samples were ground to a powder with a mortar and pestle. One (±0.1) mg of dried tissues and food sources was analysed, either by a Flash EA 1112 CN analyser coupled with a Delta Plus mass spectrometer, *via* a Conflow III interface at the Roscoff marine station (France) for the 2004 samples, or by a GV IsoPrime (UK) stable isotope mass spectrometer at Iso-Analytical (Crewe, UK) for the 2010–2012 samples. Stable isotopic data are expressed as the relative per mil (‰) differences between the samples and the conventional standard Pee Dee Belemnite for carbon and air N_2 for nitrogen, according to the following equation:

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

where X (‰) is ^{13}C or ^{15}N abundance and R is the ^{13}C:^{12}C or ^{15}N:^{14}N ratios. The typical precision in analyses was ±0.05‰ for carbon and ±0.12‰ for nitrogen.

The Bayesian mixing model implemented in the software package SIAR v.4 (Parnell *et al.* 2010) was used to estimate the relative contributions of different food sources to the diet of the eight vent gastropods described previously (Table 1, Fig. 2) using TEFs of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ (De Niro & Epstein 1978, 1981; Minagawa & Wada 1984; De Busserolles *et al.* 2009; Sweetman *et al.* 2013). Therefore, we tested several hypothetical microbial and detrital food sources (maximum of five sources per sampling year and habitat with their standard deviation when possible; Table 1). The mixing model generated robust probability estimates of source proportions taking into account natural variation and uncertainty (Parnell *et al.* 2010).

Statistical analyses

One-way analyses of variance (ANOVAs) were carried out to compare the different stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the different gastropod species studied in 2004, 2010 and 2012 after assessing normality of residuals and homoscedasticity. Significant differences were assessed by using a *post-hoc* Tukey's test (MINITAB v.15).

Results

Stable isotope signatures of the possible food sources following vent sites

The potential food sources varied following habitats of vent gastropods at EPR 9°50′ N, 12°50′ N and 17°25′ S (Table 2). Five potential food sources were generally used into the Bayesian mixing model for gastropods recovered into habitats dominated by mussel and siboglinid and three food sources were used for those recovered on alvinellid habitat. In order to be able to use these potential food sources within the mixing model, the stable isotopic signatures had to differ. For example, carbon stable-isotope signatures of biofilm scraped from *Alvinella pompejana* tubes were different from those of the episymbiont of *A. pompejana* (~10.6‰ *versus* ~8.9‰, respectively), as were those of the biofilm of the tube of *Riftia pachyptila* and the siboglinid itself at EPR 9°50′ N in 2012 (~14.7‰ *versus* ~11.2‰, respectively) and of the biofilm on the shell and the mussel itself at EPR 17°25′ S in 2004 (~−31.2‰ and ~−34.5‰, respectively) and at EPR 9°50′ N in 2012 (~−26.7‰ and ~−29.9‰, respectively). Therefore, we were able to discriminate among and use these potential food sources in the mixing models (Table 2).

Stable isotope signatures of the eight vent gastropods and food contributions following vent sites

In 2010, $\delta^{13}C$ signatures of *Lepetodrilus elevatus* differed significantly between mussel and siboglinid habitats and

Table 2. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of some potential food sources sampled from diverse habitats regarded as primary producers in this study and used in the Bayesian mixing model (sources files in SIAR).

stable isotopes in ‰	$\delta^{13}C$			$\delta^{15}N$		
sites on EPR	17°25′ S	9°50′ N		17°25′ S	9°50′ N	
habitats on vent	HBHR	HBHR	HA	HBHR	HBHR	HA
tissue of mussel	−34.5	−29.9 ± 0.5		−4.6	2.7 ± 2.2	
biofilm of mussel shell	−31.2	−26.7		−5.9	2.2	
tissue of tubeworm	−13.6 ± 0.4	−11.2 ± 0.5		1.9 ± 1.7	4.7 ± 0.5	
biofilm of tubeworm		−14.7		1.9		
tube of tubeworm		−11.5 ± 0.5		4.7 ± 0.3		
episymbiont of *Alvinella pompejana*			−8.9			6.4
biofilm of Alvinella pompejana tube			−10.6 ± 1.1			3.8 ± 0.4
particle organic matter	−27.6 ± 1.8 (Oasis) −26.4 (Rehu)	−18.8 ± 0.5 (Limén *et al.* 2007)	−12.6 ± 0.5	6.4 (Oasis & Rehu)	6.0 ± 1.4 (Limén *et al.* 2007)	4.0 ± 0.2

Data are mean and standard error of the mean (±).
HA, *Alvinella pompejana*-dominated habitat (alvinellid habitat); HBHR, habitats dominated by both siboglinid and bathymodiolin mussels; EPR, East Pacific Rise.

between EPR 9°50′ N and EPR 12°50′ N for a given habitat (ANOVA: $F_{1,2} = 258.1$; $P < 0.001$; Table 3, Fig. 3A). For *Cyathermia naticoides*, $\delta^{13}C$ values were significantly different between EPR 9°50′ N and EPR 12°50′ N sites on the siboglinid habitat (ANOVA: $F_{1,1} = 43.9$; $P < 0.001$; Table 3, Fig. 3A). However, there were no significant differences in $\delta^{15}N$ values between habitats or sites for either gastropod genus (ANOVA: $F_{1,2} = 1.8$; $P = 0.2$ for *L. elevatus*; ANOVA: $F_{1,1} = 0.03$; $P = 0.9$ for *C. naticoides*; Table 3, Fig. 3A). In the *Riftia* habitat (HR), *Riftia pachyptila* tubes were among the main contributors to the diet of *L. elevatus*: ~80% at 9°50′ N and ~38% at 12°50′ N (Fig. 3B). This was confirmed by TEM as siboglinid tubes pieces were observed alongside with bacteria (Fig. 3C and D). For *C. naticoides* in the same habitat at 9°50′ N and 12°50′ N, a similar pattern of food contribution to the diet was calculated by the mixing model (Fig. 3B) shared with a mixture of biofilm from *R. pachyptila* tubes (~27% and ~29%, respectively), tubes of the siboglinid (~30% and ~41%, respectively) and POM (~18% and ~13%, respectively). Regarding the ultrastructure of the digestive tract of *C. naticoides*, tubes of *R. pachyptila* and fungal hyphae were observed (Fig. 3E). In the *Bathymodiolus* habitat (HB) at 9°50′ N, POM, biofilm and tissue of *B. thermophilus* contributed most to the diet of *L. elevatus* (~40%, ~22% and ~21%, respectively; Fig. 3B). Electron micrographs of the digestive tract of *L. elevatus* confirmed some of these hypothetical contributions as they showed some pieces of periostracum colonized by bacteria and some possible fungal hyphae (Fig. 3F).

In 2012, at EPR 9°50′ N distinct $\delta^{13}C$ could be observed for the four gastropod species (Fig. 4A; Table 3) from the more ^{13}C-enriched to the more ^{13}C-depleted gastropod: *Peltospira operculata*, *Peltospira delicata*, *Rhynchopelta concentrica* and *Lepetodrilus pustulosus* (ANOVA: $F_{1,3} = 167.1$; $P < 0.001$). *Post-hoc* Tukey's tests showed no significant differences between the $\delta^{13}C$ values of *P. operculata* and *P. delicata*. Likewise, the $\delta^{15}N$ values of the four gastropod species were significantly different (ANOVA: $F_{1,3} = 16.8$; $P < 0.001$), except for those of *R. concentrica* and *L. pustulosus* (*post-hoc* Tukey's test; Fig. 4A). Types of food sources and their relative contribution to the diets of primary consumers varied according to habitat (Fig. 4B). In the alvinellid habitat (HA), the three contributors were the biofilm of *Alvinella pompejana*, its episymbiont and POM, making up ~25%, ~52% and ~23%, respectively, of the diet of *P. delicata*, and ~40%, ~24% and ~36%, respectively, of that of *P. operculata* (Fig. 4B). The ultrastructure of *P. operculata*'s digestive tract highlighted rod-shaped bacteria (0.6–1.4 μm) in the lumen and a few mineral particles (Fig. 4C and D). In *R. pachyptila* and *B. thermophilus* habitats (HBHR), tissues of the siboglinid *Oasisia alvinae* and POM were the main contributors to the diets of *R. concentrica* (~78% and ~14%, respectively) and *L. pustulosus* (~34% and ~34%, respectively), the contribution of the biofilm of *R. pachyptila* to the diet of the latter gastropod being ~15% (Fig. 4B).

In 2004, at EPR 17°25′ S, $\delta^{13}C$ values (Fig. 5A, Table 3) were significantly different (ANOVA: $F_{1,3} = 157.1$; $P < 0.001$) among the four gastropod species, ranging from most ^{13}C-enriched to most ^{13}C-depleted in the following order: *L. pustulosus*, *L. elevatus*, *L. ovalis* and *Eulepetopsis vitrea*. *Post-hoc* Tukey's tests showed no significant differences between the $\delta^{13}C$ values of *L. elevatus*

Table 3. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of the eight gastropods classified following habitats of *in situ* recovery.

stable isotopes in ‰	$\delta^{13}C$						$\delta^{15}N$					
years of sampling	2004	2010			2012		2004	2010			2012	
sites on EPR	17°25′ S	9°50′ N		12°50′ N	9°50′ N		17°25′ S	9°50′ N		12°50′ N	9°50′ N	
habitats on vent	HBHR	HR	HB	HR	HA	HBHR	HBHR	HR	HB	HR	HA	HBHR
Lepetodrilus elevatus	−18.8 ± 1.8	−11.3 ± 0.6	−21.0 ± 1.0	−16.0 ± 0.4			3.5 ± 0.9	7.5 ± 0.4	7.0 ± 0.2	7.9 ± 0.5		
Lepetodrilus pustulosus	−14.1 ± 1.0					−15.9 ± 0.5	5.6 ± 0.4					7.9 ± 0.2
Lepetodrilus ovalis	−21.2 ± 1.4						7.0 ± 0.6					
Eulepetopsis vitrea	−27.3 ± 1.2						5.8 ± 0.9					
Cyathermia naticoides		−9.0 ± 0.3		−10.8 ± 0.4				6.8 ± 0.5		6.8 ± 0.5		
Peltospira delicata					−9.1 ± 0.1						8.7 ± 0.3	
Peltospira operculata					−8.7 ± 0.0						6.9 ± 0.2	
Rhynchopelta concentrica						−12 ± 0.4						7.9 ± 0.2

Data are mean and standard error of the mean (±).
HA, *Alvinella pompejana*-dominated habitat; HR, *Riftia pachyptila*-dominated habitat; HB, *Bathymodiolus thermophilus*-dominated habitat; HBHR, habitat with a mixture of *Riftia pachyptila* and *Bathymodiolus thermophilus*; EPR, East Pacific Rise.

and *L. ovalis* although they were not sampled at the same sites (Oasis and Rehu Marka, respectively; Table 1) or habitats. Similarly, $\delta^{15}N$ values were significantly different (ANOVA: $F_{1,3} = 43.2$; $P < 0.001$) among the four gastropod species, but only $\delta^{15}N$ of *L. elevatus* differed from those of the three other vent gastropods using a *post-hoc* Tukey's test (Table 3, Fig. 5A). The biofilm on *Riftia* tubes and tissues of *R. pachyptila* were the main contributors to the diets of *L. pustulosus* (~64% and ~22%, respectively) and *L. elevatus* (~30% and ~33%, respectively; Fig. 5B). For *L. pustulosus*, the biofilm on mussel shells and *B. thermophilus* tissues also contributed (~17% and ~13%, respectively). POM contributed relatively little to the diet of these two limpet species compared with *L. ovalis* and *E. vitrea*, for which POM contributed ~53% and ~60% to their diet, respectively (Fig. 5B). For *L. ovalis*, the secondary sources were linked to *R. pachyptila* habitat (~16% and ~21% from biofilm and tissue, respectively), whereas for *E. vitrea*, the secondary sources were linked to *B. thermophilus* habitat (~10% and ~24% from tissue and biofilm, respectively; Fig. 5B).

Discussion

In this study we have highlighted the wide range of stable isotope ratios in carbon signatures for eight generally bacterivorous and/or detritivorous, grazing and/or filter-feeding gastropods (−30‰ to −8‰), and determined the relative contributions of some potential food sources to their diets by using a Bayesian mixing model. We have sampled several potential food sources but were constrained to use only a few sources when running the mixing model (Boecklen *et al.* 2011), although alternate sources may exist. The study has highlighted that at the base of a vent food web there are abundant and isotopically variable food sources issued from numerous microbial pools, numerous bacterial invertebrate symbioses and an accumulating detrital pool. The relevance of any of these sources within a mixing model has to be carefully evaluated against the spatial niche of the targeted species for which we want to reconstruct the diet.

Microbial chemoautotrophic producers at vents and metabolism

Three isotopically distinct microbial chemoautotrophic sources of primary producers were highlighted (but not measured) by Bergquist *et al.* (2007). Some authors (Van Dover & Fry 1994; Colaço *et al.* 2002) measured the $\delta^{13}C$ and $\delta^{15}N$ of several bacterial mats but again the phylogenetic origins of the bacteria were not ascribed. To our knowledge, the only isotopic signature known prior to the present study is that of the dominant group of

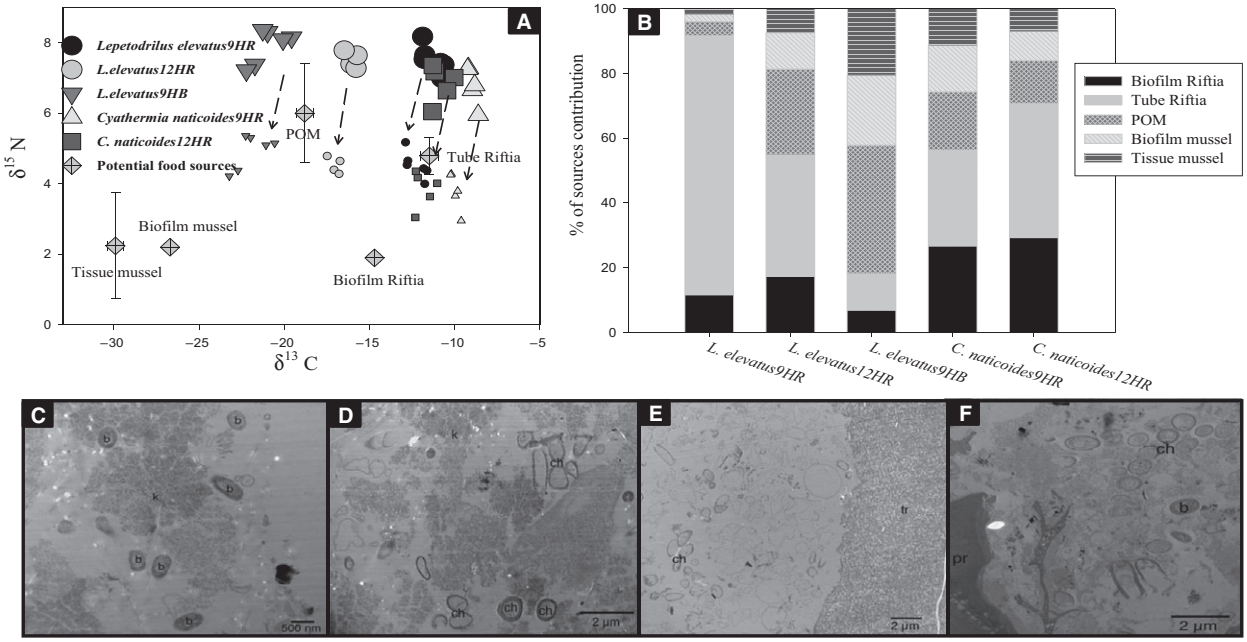

Fig. 3. Synthesis of the diet reconstruction of *Lepetodrilus elevatus* and *Cyathermia naticoides* sampled at East Pacific Rise (EPR) 9°50′ N and EPR 12°50′ N in 2010. (A) Plot in two dimensions of $\delta^{13}C$ and $\delta^{15}N$ values of the two vent gastropod species and their potential food sources sampled in habitats dominated either by *Bathymodiolus thermophilus* (HB) or *Riftia pachyptila* (HR). The smaller symbols are the corrected stable isotopic values using trophic enrichment factors of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ and error bars are the standard deviations of the mean when possible. (B) Contributions of potential food sources (details in Tables 1 and 2) calculated using the Bayesian mixing model stable isotope analysis in R (SIAR) v. 4 (Parnell *et al.* 2010). (C–F) Ultrastructure of digestive tracts of the two gastropod species (Table 1). (C and D) Content of digestive tract from *L. elevatus* collected from siboglinid habitat showing pieces of degraded tubes of *R. pachyptila*, evidenced by chitin (k), bacteria (b) and fungal hyphae (ch). (E) Content of digestive tract from *C. naticoides* collected from siboglinid habitat showing pieces of *R. pachyptila* tubes (tr) and fungal hyphae (ch). (F) Content of digestive tract from *L. elevatus* collected from mussel habitat showing pieces of periostracum (pr) colonized by bacteria (b) and fungal hyphae (ch). POM, particulate organic matter.

Epsilonproteobacteria (using primarily the rTCA pathway), in which $\delta^{13}C$ values are between −12‰ and −8‰ (Campbell *et al.* 2006; Sievert & Vetriani 2012).

In this study, by using the stable isotope signatures of the symbiotic megafauna as proxies, three distinct chemoautotrophic microbial sources were indirectly measured. First, the carbon isotopic signature of the episymbiont of *Alvinella pompejana* (~−9‰) matched the ^{13}C-enriched value previously described in alvinellid habitat of free-living *Epsilonproteobacteria* (Campbell *et al.* 2006; Sievert & Vetriani 2012). The second main group of free-chemoautotrophic bacteria is made up of the *Gammaproteobacteria*, which are closely related to endosymbionts that live in the trophosome of siboglinids and use the CBB cycle as a carbon fixation pathway with form II RubisCO (Robinson & Cavanaugh 1995). All carbon stable isotope ratios from siboglinid tubeworms measured in this study, *i.e.* tissue of *Oasisia alvinae* in 2012 (~−11.2‰), tissue of *Riftia pachyptila* in 2004 (~−13.5‰) and tubes of *R. pachyptila* in 2010 (~−11.5‰), matched those from chemoautotrophic bacteria in trophic web studies (~11‰) (in Limén *et al.* 2007) and also fall within the '−11‰' range reported by Robinson & Cavanaugh (1995). Recently, it was identified that the endosymbiont of *R. pachyptila* (*Candidatus* 'Endoriftia persephone'; Robidart & Bench 2008) is able to use the rTCA pathway as an alternative carbon fixation pathway using the key enzyme ATP citrate lyase (Gardebrecht *et al.* 2012). Free-living chemoautotrophic bacteria from mussel habitats have carbon stable isotope signatures close to the isotopic composition of the tissue of *Bathymodiolus thermophilus*, which harbors endosymbionts within bacteriocytes in its gills (~34.5‰ in 2004 and ~30‰ in 2012). These values fall into the '−30‰' group of chemoautotrophic bacteria using form I RubisCO (Robinson & Cavanaugh 1995).

Overall, the $\delta^{13}C$ signatures of these proxies of chemoautotrophic prokaryotes were most enriched in ^{13}C at the sites closest to the vent openings (−9‰) and most depleted at those furthest away from the vent openings (−34.5‰), in line with previous studies (Levesque *et al.* 2005; Limén *et al.* 2007). According to those studies, the isotopic signatures of potential chemoautotrophic bacteria

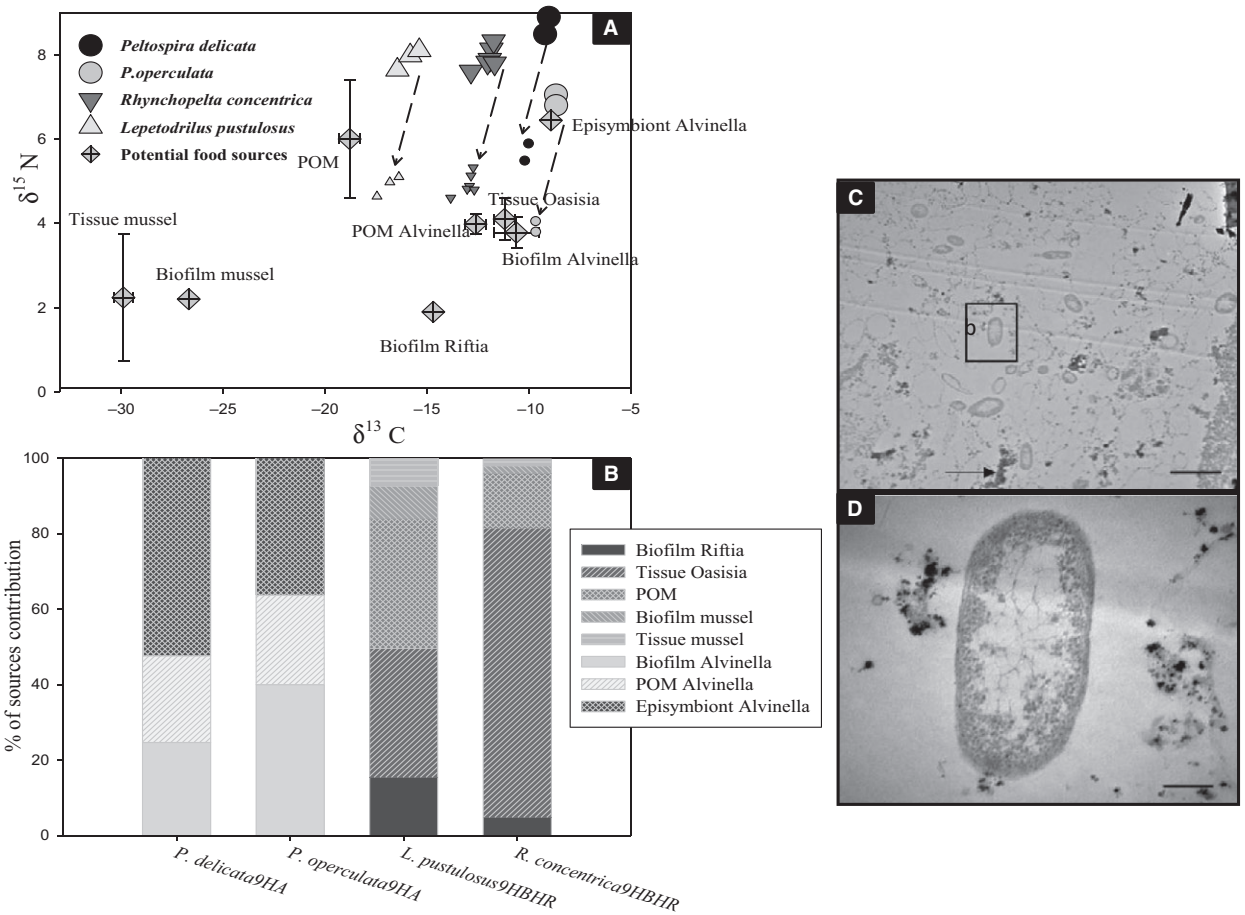

Fig. 4. Synthesis of the diet reconstruction of *Lepetodrilus pustulosus*, *Rhynchopelta concentrica*, *Peltospira delicata* and *Peltospira operculata* sampled at East Pacific Rise 9°50′ N in 2012. (A) Plot in two dimensions of $\delta^{13}C$ and $\delta^{15}N$ values of the four vent gastropod species and their potential food sources sampled in habitats dominated either by *Alvinella pompejana* (HA) or by both *Bathymodiolus thermophilus* and *Riftia pachyptila* (HBHR). The smaller symbols are the corrected stable isotopic values using trophic enrichment factors of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ and error bars are the standard deviations of the mean when possible. (B) Contributions of potential food sources (details in Tables 1 and 2) calculated using the Bayesian mixing model stable isotope analysis in R (SIAR) v. 4 (Parnell *et al.* 2010). (C and D) Ultrastructure of digestive tracts of *P. operculata* (Table 1). (C) Content of digestive tract showing rod-shaped bacteria (b) and few mineral particles (arrow). (D) Close-up of one of the rod-shaped bacteria. Scale bars = 2 μm (C) and 185 nm (D). POM, particulate organic matter.

become more depleted in ^{13}C away from the vent openings as the dissolved inorganic carbon (DIC) of the seawater is more depleted than that of the vent fluid.

Other microbial producers at vents

Microbial diets other than the one based solely on chemoautotrophy at hydrothermal vents may consist of complex microbial biofilms that have colonized the surfaces provided by the foundation species (Govenar 2010), such as the proteic tubes of *Alvinella pompejana*, the chitinous tubes of *Riftia pachyptila* and the shells of *Bathymodiolus thermophilus*. The nature of the biofilms recorded in the present study was not analysed but previous work on tubes of *R. pachyptila* sampled at EPR 9°50′ N and 12°50′ N indicate that these biofilms comprise a mixture of heterotrophic and autotrophic bacteria: not only *Alpha-*, *Delta-*, *Epsilon-* and *Gammaproteobacteria*, but also Verrucomicrobia and Bacteroidetes (Lopez-Garcia *et al.* 2002). Burgaud *et al.* (2009) managed to isolate and cultivate different strains of filamentous fungi scraped from biological hydrothermal vent samples, such as tubes of *A. pompejana* from EPR and shells of *Bathymodiolus azoricus* from the Mid-Atlantic Ridge. Therefore, it is probable that fungi which seem to be distributed ubiquitously at vents (Burgaud *et al.* 2009; Le Carvez *et al.* 2009), were present in the biofilms used in this present study. Fungi were also present within the gut content of *Lepetodrilus elevatus* sampled from both mussel and siboglinid habitats as fungal hyphae were observed

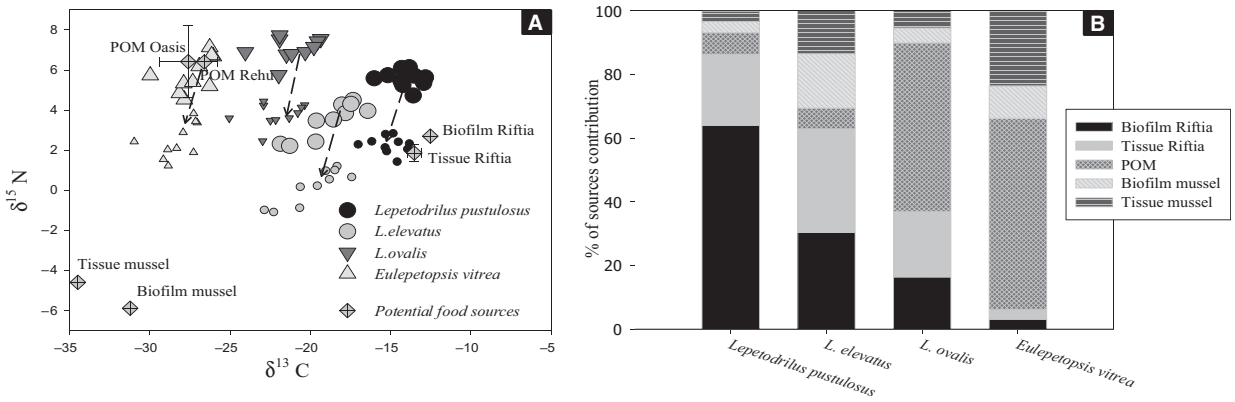

Fig. 5. Synthesis of the diet reconstruction of *Lepetodrilus pustulosus*, *Lepetodrilus elevatus*, *Lepetodrilus ovalis* and *Eulepetopsis vitrea* sampled at East Pacific Rise 17°25′ S in 2004. (A) Plot in two dimensions of $\delta^{13}C$ and $\delta^{15}N$ values of the four vent gastropod species and their potential food sources sampled from habitats dominated by both *Bathymodiolus thermophilus* and *Riftia pachyptila*. The smaller symbols are the corrected stable isotopic values using trophic enrichment factors of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ and error bars are the standard deviations of the mean when possible. (B) Contributions of potential food sources (details in Tables 1 and 2) calculated using the Bayesian mixing model stable isotope analysis in R (SIAR) v. 4 (Parnell *et al.* 2010). POM, particulate organic matter.

using TEM. Despite being found within the digestive tract of primary consumers, it is not known whether fungi have any nutritional function to these consumers.

Detrital production at vent following habitats

Although POM was not sampled in all habitat types on vent fields in the present study, we confirmed previous observations that POM carbon isotopic signatures vary spatially and are enriched in ^{13}C close to vent openings and depleted in ^{13}C further away. For example, at EPR 17°25′ S, two samples that displayed very depleted ^{13}C signatures (−26.4‰ and −27.6‰) were obtained from a vent mussel bed. The highly depleted stable isotope values of the POM from mussel habitat may result from a combination of chemosynthetic sources issued from the mussel bed (the '−30‰' group, Robinson & Cavanaugh 1995) and photosynthetically derived surface material. At EPR 17°25′ S, at 80 m below the ocean surface, the $\delta^{13}C$ value was −24‰ (Thiébaut and Riera unpublished data). At both EPR 9°50′ N and EPR 12°50′ N for *Riftia pachyptila* habitat, we used the POM value measured by Limén *et al.* (2007) for similar hydrothermal fluid conditions and proximity to vent openings on the chimney wall. This value (~18.8‰), depleted in ^{13}C, was the result of a mixture of chemosynthetic sources issued from the siboglinid habitat (the '−11‰' group, Robinson & Cavanaugh 1995) and photosynthetically derived surface material, but was still more enriched when compared with the $\delta^{13}C$ of POM from colder vent habitats, such as bathymodiolin mussel beds. At EPR 9°50′ N, stable isotope signatures of POM were also measured in water samples from the alvinellid habitat. This value (~12.6‰) was more enriched in C^{13} compared with POM from the siboglinid and bathymodiolin mussel habitats, but still more depleted compared with the isotopic signature of the *Epsilonproteobacteria* that dominate this part of the chimney wall (Campbell *et al.* 2006; Sievert & Vetriani 2012).

Use of sources by primary consumers

In a linear model, when the number of sources exceeds the number of equations (number of isotopes + 1), the system of equations is undetermined (Boecklen *et al.* 2011). Here, using a Bayesian mixing model (Parnell *et al.* 2010) we were able to calculate some contribution up for five sources, allowing greater flexibility than a classical two-source mixing model, such as that used by Limén *et al.* (2007), which included chemolithoautotrophic bacteria and POM only. Bayesian mixing models can thus be used to solve some undetermined systems of equations common in mixing models that others models cannot (Parnell *et al.* 2010). There is some disagreement about this solution (see review by Boecklen *et al.* 2011) and some authors have suggested that Bayesian mixing models are just an alternative to the IsoSource family of models (Philipps and Gregg 2003). In this study, one or two major sources generally constituted most of the diet for a given gastropod species, with the others being less important. When the number of sources was higher than five sources (data not shown), the solution became less robust. When using mixing models with multiple sources, it is mandatory to know where the species were sampled, as isotopic signatures of potential food sources (POM, biofilms and chemoautotrophic bacteria) and relevant

sources to be included may vary greatly depending on their proximity to vent openings as discussed previously.

The $\delta^{15}N$ values of the eight gastropod species place most of them at their expected trophic level, *i.e.* that of primary consumers with signatures between 6‰ and 8‰, with the exception of *Lepetodrilus elevatus* sampled at EPR 17°25′ S in which the $\delta^{15}N$ values were much lighter (~2 to 4‰) compared with conspecifics from EPR 9°50′ N and EPR 12°50′ N (~7 to 8‰; Table 3). Sweetman *et al.* (2013) measured very light $\delta^{15}N$ values in the rissoid gastropod *Pseudosetia griegi* and, using a mixing model, demonstrated that this gastropod's diet relied mostly on grazing upon SOX bacteria. The differing relative contributions of microbial and detrital sources to the diets of *L. elevatus* specimens from different populations sampled at EPR 17°25′ S, EPR 9°50′ S and EPR 12°50′ N may explain the low $\delta^{15}N$ values for individuals from the former field because of a reduced POM contribution and a greater biofilm contribution to their diets.

It is commonly suggested that invertebrates hosting symbionts (*e.g.* siboglinids and bathymodiolin mussels), despite representing a high biomass at vents, do not directly confer nutrition to non-chemosymbiotic species in the general vent food web (Fisher *et al.* 1994; Bergquist *et al.* 2007; De Busserolles *et al.* 2009; Govenar 2012), despite observations *in situ* (Bright & Lallier 2010) reporting predatory behavior on the plumes of tubeworms. Here, using a mixing model, we demonstrated that some gastropods derive part of their nutrition from both biofilms and 'tissues' of tubeworms (*L. elevatus*, *Lepetodrilus pustulosus*, *Lepetodrilus ovalis*, *Cyathermia naticoides*, *Rhynchopelta concentrica*) and mussels (*L. elevatus*, *Eulepetopsis vitrea*). Although pieces of siboglinid tubes were observed in the guts of *C. naticoides* and of *L. elevatus* (as seen in *L. ovalis* and *L. pustulosus* by Fretter 1988), and pieces of mussel periostracum in *L. elevatus*, these were probably ingested when grazing on bacteria, as these species are neither carnivores nor scavengers, supported by low $\delta^{15}N$ values. This is also supported by Fretter's (1988) observations of feces from *L. pustulosus* that contained some cleaned scrapes of *Riftia* tubes. He described *Lepetodrilus* species as possessing the feeding apparatus of both filter-feeders and of grazers, having modified frontal pads on lamellar tips and a radula that is not rachiglossate (Fretter 1988). Likewise, *C. naticoides* is also suspected to be a filter-feeder (Desbruyères *et al.* 2006). This supports our hypothesis that these gastropods graze on free-living chemoautotrophic bacteria (or free-living forms of symbionts) that have stable isotope signatures close to those of the tissues of metazoan hosting endosymbionts. Thus, we can use the latter as proxies for these free-living chemoautotrophic bacteria. At EPR 17°25′ S, we distinguished between the first group, consisting of *L. elevatus*, *L. pustulosus* and *L. ovalis*, which grazed preferentially on *Gammaproteobacteria* and used the CBB cycle and form II RubisCO, and the second group, including *E. vitrea*, which grazed preferentially on *Gammaproteobacteria* with a CBB cycle and form I Rubisco. According to Fretter (1990), *E. vitrea* does not harbor any gills in its palleal cavity and has a docoglossate radula. At EPR 9°50′ N and 12°50′ N, we differentiated specimens of *L. elevatus* from siboglinid habitat, which grazed preferentially on *Gammaproteobacteria* with form II RubisCO, from their conspecifics on mussel-bed habitat, which grazed preferentially on *Gammaproteobacteria* with form I RubisCO. As previously proposed by Desbruyères & Segonzac (2006), we confirmed that *C. naticoides* can be both a filter-feeder, as POM may figure in the diet at both EPR 9°50′ N and 12°50′ N, and an active grazer, feeding on *Gammaproteobacteria* with form II RubisCO. At EPR 9°50′ N, *Peltospira delicata* and *Peltospira operculata* were both grazers on *Epsilonproteobacteria* and filter-feeders on POM and possessed a rhipidoglossate radula (Fretter 1989). *Rhynchopelta concentrica* and *L. pustulosus* grazed preferentially on *Gammaproteobacteria* with form II RubisCO.

Food partitioning and trophic niche

As already shown in previous studies of reducing habitats with alvinellid (Levesque *et al.* 2003) and dorvilleid species (Levin *et al.* 2013), food partitioning occurs between different species of the same or closely related genera, allowing organisms to co-exist by occupying distinct trophic niches in a biological community, and decreasing competition for resources. However, not all species have distinct trophic niches and some may overlap. In this study, food partitioning between limpets of the genera *Lepetodrilus* and *Eulepetopsis* was evident based on specimens collected at EPR 17°25′ S. Only *Lepetodrilus elevatus* and *Lepetodrilus pustulosus* shared very similar niches, and possibly competed with one another, while others displayed distinct carbon stable isotope signatures. At EPR 9°50′ N, *L. elevatus* and *L. pustulosus* were most abundant in the siboglinid habitat, whereas *Eulepetopsis vitrea* and *Lepetodrilus ovalis* occurred mostly in the mussel habitat (Mills *et al.* 2007). At EPR 17°25′ S, biofilm from *Riftia pachyptila* tubes dominated the diet of *L. pustulosus* (~60%) and to a lesser degree that of the sympatric species *L. elevatus* (~30%). POM dominated the diet of *L. ovalis* and *E. vitrea*. The second main source differed between the two species, with *Gammaproteobacteria* using the CBB cycle with form I RubisCO in *E. vitrea*, and *Gammaproteobacteria* with form II RubisCO in *L. ovalis*. Here, the two groups of gastropods used different principal microbial or detrital sources, and

within these two groups, the relative contribution of each differed, permitting food partitioning among species, within habitats and between habitats.

Food partitioning between different species not only differed between habitats at vent on a particular vent chimney, but also at the microhabitat level. At both EPR 9°50′ N and EPR 12°50′ N, *L. elevatus* and *C. naticoides* were sampled on the tubes of *R. pachyptila*. In both locations, carbon stable isotope signatures of the two species were distinct (most obvious for EPR 12°50′ N; Fig. 3A). Despite being sympatric, they displayed distinct sub-habitat preferences. Mills *et al.* (2007) have shown that The neomphalid gastropod aggregated at the base of the *R. pachyptila* tubes whereas the lepetodrilid aggregated higher up the tubes. The co-existence of these two species might be possible because they share similar food sources but consume them in different proportions; the Bayesian mixing model revealed that *C. naticoides* contained a higher proportion of biofilm originating from *R. pachyptila* tubes compared with *L. elevatus*. At EPR 9°50′ N, *C. naticoides* also seemed to forage on mussel beds, reflecting some dietary contribution from the mussel habitat's microbial pool. This species may extend its trophic niche to avoid being in competition with sympatric species.

Specialists, generalists

At EPR 9°50′ N in 2012, food partitioning seemed to have occurred between the peltospirid genera *Rhynchopelta* and *Peltospira*, which display different trophic niches, but not within the genus *Peltospira* itself. *Peltospira delicata* and *Peltospira operculata* shared the same resources in more or less the same relative proportions, and therefore may have been in direct competition. These two species are specialists according to the definition of Bearhop *et al.* (2004) because the variance in the stable isotope ratios of the tissue was very low to null. In the present study, these two species were found in the alvinellid habitat in stressful conditions close to the vent opening (greater temperature and higher sulfide concentration compared with locations further from the vent opening). A narrow range of stable isotope ratios of invertebrates in this habitat at EPR 9°50′ N was previously shown in a colonization experiment and interpreted as being the result of the limited choice of dietary resources available (Gaudron *et al.* 2012).

Some species at vents are truly generalists (the variance of the stable isotope ratios of their tissues is large for a given species according to Bearhop *et al.* 2004). They are able to switch diet, as is the case for some alvinellid polychaetes at the Juan de Fuca Ridge (Levesque *et al.* 2003). In our study, *Lepetodrilus elevatus* is a true generalist species as defined by Bearhop *et al.* (2004), as carbon stable isotope ratios from EPR 9°50′ N ranged from −22.2‰ to −10.6‰, with a variance of 5.2‰ (n = 12). The Bayesian mixing model revealed a diversity of resources among the different specimens including POM, biofilms of mussels and *Gammaproteobacteria* with form I and II RubisCO. *Lepetodrilus elevatus* at EPR 9°50′ N has a wide trophic niche, being more bacterivorous on siboglinid habitat than on mussel habitat. Similarly, at EPR 17°25′ S, carbon stable isotope ratios of *L. elevatus* ranged from −21.8‰ to −16.4‰ with a variance of 1.8‰ (n = 10). Applying the mixing model to these specimens revealed that *L. elevatus* had been feeding on the microbial pools of both the siboglinid and the mussel habitats. This species was recovered in colonization experiments deployed at EPR 9°50′ N following the eruption in 2006, at high densities in those deployed on siboglinid habitat and at lower densities in those deployed on basalt and alvinellid habitats (Gaudron *et al.* 2012). This suggests wide thermal or sulfidic tolerances compared with the other three species (Mills *et al.* 2007; Matabos *et al.* 2008a), allowing the expansion of its trophic niche, and can be regarded as an example of ecological release as seen in some dorvilleid polychaetes from cold seeps (Levin *et al.* 2013). Such a release would allow the species to extend its spatial habitat, access additional nutritional resources and decrease intra- and inter-specific competitive pressure.

Conclusions

Gastropods at hydrothermal vents are primary consumers, and feed mainly on microbes and POM. Intra-specific (*e.g. Lepetodrilus elevatus*) and inter-specific resource partitioning (*e.g.* within lepetodrilids) was identified, whereby some species were able to switch diet when necessary, possibly limiting intra- or inter-specific competition. Several microbial and detrital sources exist at vents and display distinct isotopic signatures, emphasizing the need to apply multi-source mixing models to calculate their relative contributions to the diets of primary consumers. Here, six microbial sources were identified as having distinct stable isotope signatures: proxies of *Gammaproteobacteria* form I RubisCO, of *Gammaproteobacteria* form II RubisCO and of *Epsilonproteobacteria*, and biofilms of siboglinid tubes, of alvinellid tubes and of mussel shells. Three detrital sources were identified as having distinct stable isotope signatures in the different vent habitats. Besides heterotrophy, symbiosis may occur within the gills of these small gastropods. Given that symbionts would most likely belong to the same groups represented in the food sources, their dietary contribution could not be directly tested here, and should be investigated using alternate approaches. Finally, tagged

gastropods could also be followed *in situ* by video survey, to confirm their foraging behavior and eventual small-scale spatial niche partitioning.

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