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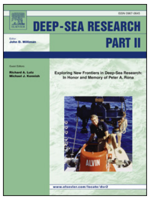

# Variation in the diets of hydrothermal vent gastropods

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## ABSTRACT

A prevailing paradigm of hydrothermal vent ecology is that primary consumers feed on chemoautotrophic bacteria. However, for the purposes of reconstructing vent food webs and for tracking energy flow from the generation of rock and fluid chemistry through primary/ secondary productivity and consumption to the overlying water column, it remains unclear which consumers feed on which bacteria. In paired analyses of carbon and nitrogen tissue stable isotope values with unique 16S rRNA sequences from the stomach contents, we determined that two species of gastropod grazers appear to feed on epsilon-proteobacteria, while two other species have more diverse diets, including one species that consumes alpha-proteobacteria, planctomycetes, and non-green sulfur bacteria. Different carbon fixation pathways used by epsilon- and alpha-proteobacteria may account for the variation in the carbon stable isotope values among the consumers. Furthermore, our results indicate that trophic specialization and niche partitioning may contribute to the distribution and abundance of vent-endemic gastropods and support the hypothesis that consumers in the warmer habitats commonly feed on epsilon-proteobacteria that use the rTCA cycle, while in the cooler habitats they feed on additional bacteria that use the CBB cycle. These results suggest that the phylogenetic and metabolic diversity of free-living bacteria may play an important and previously overlooked role in facilitating species coexistence among primary consumers at hydrothermal vents and other chemosynthesis-based ecosystems.

## 1. Introduction

While a number of spatially-dominant species in hydrothermal vent communities have symbiotic relationships with chemoautotrophic bacteria, the majority of endemic vent metazoans are consumers that apparently do not feed on symbiont-containing megafauna (Fisher et al., 1994; Bergquist et al., 2007; De Busserolles et al., 2009). Most vent invertebrates are grazers and deposit feeders that consume free-living microbes, protists, and other small invertebrates (Van Dover and Fry, 1989; Levesque et al., 2005; Limén et al., 2007). A few specific trophic relationships have been identified among protists (Saudevet et al., 2010), meiofauna (Limén et al., 2007, 2008), and macrofauna (e.g., Fisher et al., 1994; Sancho et al., 2005; Voight, 2005; Bergquist et al., 2007; Voight and Sigwart, 2007; (De Busserolles et al., 2009), but the vast majority of the relationships are undefined at the base of the food web.

Although chemosynthetic primary producers are found in the subsurface biosphere on and near the seafloor, in the vent effluent, and in the hydrothermal plume, metazoan consumers are more or less confined to the seafloor and overlying water column (Govenar, 2012). Within this smaller range of vent habitats, many studies have identified two or more isotopically distinct microbial food sources for consumers (e.g., Van Dover and Fry, 1994), but surprisingly little is known about which chemosynthetic microbes are consumed. In a survey of lipid profiles from different consumers from diffuse-flow vents at the East Pacific Rise, bacteria appeared to be the primary food source (Phleger et al., 2005a, 2005b). However, neither functional nor phylogenetic groups of bacteria could be distinguished, and the neither the consumption of archaea nor archaeal lipids could be confirmed (Thurber et al., 2012). Archaea may be consumed where they are more prevalent, such as high-temperature chimneys and vents with high methane concentrations, where there is a higher incidence of methanogens (Takai et al., 2006; Thurber et al., 2012). Heterotrophic bacteria, abiotic organics, and photosynthetic detritus can also be important sources of organic carbon in areas of lower hydrothermal fluid flux (Tunnicliffe et al., 2003; Levesque et al., 2005; Limén et al., 2008).

Gastropods are the most abundant and diverse grazers at deep-sea hydrothermal vents (Desburyères et al., 2006) and could play an important role in structuring vent communities and the flow of energy through top-down processes that regulate primary production and metazoan recruitment. Over 94,000 *Depressigyra globulus* individuals and 33,800 *Lepetodrilus fucensis* were recovered from a single

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aggregation of the tubeworm *Ridgiea piscesae* (Bergquist et al., 2007), and $>$38,900 individuals representing 13 different gastropod species were collected with a single aggregation of the tubeworm *Riftia pachyptila* (Govenar et al., 2005). Some gastropod species are widely distributed among different hydrothermal vent habitats, and others occur in specific microhabitats, in association with particular foundation species, or during some stages of succession (Van Dover, 2002; Mullineaux et al., 2003; Govenar et al., 2005; Mills et al., 2007). Experiments have demonstrated that some vent-endemic gastropods are capable of actively seeking warmer temperatures, presumably to take advantage of greater food availability (Bates et al., 2005). Other species occupy cooler habitats (Bates et al., 2005; Mills et al., 2007) in response to abiotic or biogenic cues or to avoid predators in warmer zones (Mills et al., 2007). While morphological evidence, microscopic analysis of stomach contents, stable isotopes, and lipid profiles of gastropods suggest that gastropods feed primarily on free-living bacteria (e.g., Fretter, 1988, 1990; Fisher et al., 1994; Phleger et al., 2005a, 2005b), the results of manipulative field experiments suggest that some of the same gastropod species may also be grazing on invertebrate recruits and substantially altering macrofaunal community structure (Micheli et al., 2002; Mullineaux et al., 2003). In addition to the ecological implications of consuming either primary producers or invertebrate recruits, differences in the diets of co-occurring species may contribute to the high abundance and diversity of vent gastropods through niche partitioning.

Habitat specialization among co-occurring vent gastropod species may drive differences in their diets, resulting in ecological or evolutionary trade-offs that facilitate species coexistence. For example, the patellomorph (limpet) gastropod *Lepetodrilus elevatus* is numerically dominant nearly everywhere it is sampled (Dreyer et al., 2005; Govenar et al., 2005; Mills et al., 2007), but it occurs in higher relative abundance in areas where there are more concentrated hydrothermal fluids (Mills et al., 2007). A congener, *Lepetodrilus ovalis* occurs with *L. elevatus* nearly everywhere where it is sampled (Dreyer et al., 2005; Govenar et al., 2005; Mills et al., 2007), but it is relatively more abundant in areas where there are less concentrated hydrothermal fluids. Thus, gastropods (e.g., *L. elevatus*) may benefit from greater access to high productivity by chemosynthetic primary producers in warmer vent microhabitats, but may also suffer energetic costs resulting from physiological adaptations to extreme or variable temperatures, reduced chemical concentrations, and oxygen availability. Gastropods that either do not have these adaptations or select cooler habitats for other reasons (Mills et al., 2007) may feed in less productive habitats where different groups of bacteria occur. Therefore, the differences in diet stemming from habitat specialization may facilitate species coexistence in heterogeneous vent habitats and contribute to the wide distribution, high abundance, and high diversity of gastropods in hydrothermal vent communities.

To examine the variability of the diet among gastropod grazers at the East Pacific Rise and to investigate the role of trophic dynamics in structuring vent communities, we compared the tissue carbon and nitrogen stable isotope values from multiple individuals of five different species of patellomorph gastropods collected at five diffuse-flow vent sites on the East Pacific Rise (EPR) in 2002 and 2006. We also examined unique 16S rRNA sequences (haplotypes) from the stomach contents of a subset of these gastropods collected in 2006 to identify their bacterial food sources and link specific microorganisms to the stable isotopic composition of the consumer.

## 2. Methods

Five species of gastropods were collected with the DSV *Alvin* from five diffuse-flow hydrothermal vent sites near 9°50′N on the East Pacific Rise (Table 1). In 2002, gastropods were collected from four diffuse-flow vent sites, either with intact aggregations of *R. pachyptila*, using the Bushmaster Jr. (as described in Govenar et al. (2005)) or with scoops of *Bathymodiolus thermophilus*. In association with the gastropods sampled for this study, Le Bris et al. (2006) characterized the temperature and chemistry of diffuse-flow fluids of the four sites ("Tica", "Riftia Field", "Mussel Bed", and "Biovent"), and Govenar et al. (2005) characterized the composition of the epifaunal community associated with the aggregations of *R. pachyptila* at Tica and Riftia Field (summarized in Table 2). The community associated with *B. thermophilus* was sampled at Mussel Bed and Biovent in 2001 and characterized by Dreyer et al. (2005), which provided a valuable comparison to our samples (Table 2). In 2006, gastropods were collected with a slurp sampler from a fifth diffuse-flow vent site, "Marker 7" (9°47.26′N, 104°16.98′W), which had been spared by the 2005–06 seafloor eruption in the same area. At the time of sampling, *B. thermophilus* mussels visually dominated the megafaunal community, overgrowing many of the remaining individuals of *R. pachyptila* at the site.

**Table 1**
Collection location of the five gastropod species. All individuals were analyzed for tissue stable isotope content. Samples from Marker 7 (collected in 2006) were also analyzed for bacterial stomach content analysis.

| | Tica | Riftia Field | Mussel Bed | Biovent | Marker 7 |
|---|---|---|---|---|---|
| *Eulepetopsis vitrea* | | | 3 | 3 | 4 |
| *Lepetodrilus elevatus* | 3 | 3 | 3 | 3 | 3 |
| *Lepetodrilus ovalis* | | 3 | 3 | 3 | 2 |
| *Lepetodrilus pustulosus* | 3 | 3 | | | 3 |
| *Rhyncopelta concentrica* | 3 | 3 | | | |

The gastropods sampled for this study were immediately preserved in 95% ethanol upon recovery to the ship and then transferred to either 70% or 95% ethanol for storage and shipping to home laboratories. Then, individuals collected in 2002 were removed from their shell, dried at 60 °C, and pulverized for carbon and nitrogen stable isotope analysis at the Stable Isotope Laboratory of the Ecosystems Center, Marine Biological Laboratory. After shipping to Woods Hole Oceanographic Institution (WHOI), the gastropods collected in 2006 were dissected following ethanol-flame sterilization techniques, and both the dissected stomachs and a sample of the mantle were placed immediately into separate tubes containing ATL buffer for DNA extraction with the DNeasy Blood and Tissue kit (QIAGEN, following the manufacturer's instructions). Stomachs were easily identified in the visceral cap after removing the shell and remained intact until placed into the buffer. DNA was also extracted, following the same procedures, from the mantle and from a sample of cyanobacteria for negative and positive controls, respectively. PCR was used to amplify the entire 16S rRNA gene (1540 bp) in DNA obtained from the dissected stomachs and the controls, with universal bacterial primers (EUB1-EUB2, Ward et al., 2000). Amplicons were then purified with the QIAquick PCR purification kit (QIAGEN) and cloned with the TOPO-TA cloning kit (Invitrogen). DNA sequencing with M13-forward and M14-reverse primers was then used in one-eighth format sequencing reactions using Big Dye terminators (version 3, Perkin-Elmer) in 96-well plates and purified by isopropanol precipitation before sequencing on an ABI 3730XL capillary sequencer at The Josephine Bay Paul Center, Marine Biological Laboratory. The remains of these animals were then processed for stable isotope analysis as described above.

Carbon and nitrogen stable isotope values were tested for normal distribution, and ANOVA was used to determine significant differences among sites and among species. Pairwise comparisons of sites and species were evaluated with the Tukey Kramer test. 16S rRNA sequences obtained from the clone libraries isolated from the stomachs of each of the four gastropod species were