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## Trophic ecology of *Rimicaris exoculata*: a combined lipid abundance/stable isotope approach

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**Abstract** The alvinocaridid shrimp *Rimicaris exoculata* is an abundant component of the biota of Mid-Atlantic Ridge hydrothermal vents. To determine the nutritional strategy of this organism, we analysed the molecular abundance and carbon isotopic composition of its phospholipid fatty acids. High abundances of *n*-7 fatty acids (>40% total fatty acids) were observed in *R. exoculata* muscle tissues, in bacterial epibionts scraped from its gill bailers, and from the bacterially infested metal sulphides that the shrimp ingest. The phospholipid fatty acid abundance data indicates that the bacteria in the sulphides are closely related to the bacterial epibiota inhabiting the shrimp gill bailers, carapace and other body parts. Compound specific  $\delta^{13}\text{C}$  analyses of the phospholipid fatty acids gave average values of  $-12\text{‰}$  for the epibiont bacteria and  $-21\text{‰}$  for the sulphide bacteria. This difference may be largely due to the ex-

pression of different forms of RuBisCO (Forms I and II) which fractionate against  $^{13}\text{C}$  to different extents. Carbon limitation within the shrimp epibiont population may be an additional factor. The  $\delta^{13}\text{C}$  values (mean =  $-13\text{‰}$ ) of the saturated and monounsaturated fatty acids isolated from the muscle tissues of *R. exoculata* were very close to those of the epibionts, indicating that the predominant source of dietary carbon for the shrimp is their epibionts, with a lesser contribution from free-living bacteria. The  $\delta^{13}\text{C}$  values ( $-26\text{‰}$ ) of shrimp cholesterol were much more negative than those of the fatty acids, and this cholesterol is likely to have derived from the oceanic photic zone.

### Introduction

The alvinocaridid shrimp *Rimicaris exoculata* dominates the fauna of the Mid-Atlantic Ridge hydrothermal vent sites at 23°N (Snake Pit) and 26°N (Trans-Atlantic Geotraverse, TAG) (Segonzac et al. 1993; Van Dover 1995). These organisms form exceptionally dense clusters of up to 50 individuals per litre on sulphide mounds associated with active venting (Van Dover et al. 1988; Segonzac et al. 1993). Such an extraordinarily high biomass in a deep-sea ecosystem, together with an unusual clustering behaviour, has attracted much interest in the nutritional strategy of *R. exoculata*. These shrimp have high abundances of epibiotic bacteria beneath their carapaces and upon other body parts, but also ingest large amounts of metal sulphides scraped from the sides of the mounds (Segonzac et al. 1993). The *R. exoculata* epibiota have been identified as a monoculture and have been demonstrated to be genetically similar to the major microbial component living by oxidation of metal sulphides and incorporation of  $\text{HCO}_3^-$  (Polz and Cavanaugh 1995). Therefore, the question arises as to whether the main source of the shrimps' dietary carbon derives from epibiotic bacteria [a hypothesis put forward by Gebruk et al. (1993) and by Segonzac et al. (1993)], or

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from bacteria associated with the ingested metal sulphides (as originally suggested by Van Dover et al. 1988), or indeed a combination of the two.

Because of the difficulty of observing deep-sea organisms both in situ and in vitro, indirect methods are needed to help determine the sources of dietary carbon for such organisms. The structure (e.g. chain length and degree of unsaturation) and relative abundance of fatty acids, have been used to infer trophic relationships of a variety of hydrothermal organisms (Ben-Mligh et al. 1992; Fullarton et al. 1995; Rieley et al. 1995). Such an approach can give information upon the relative proportions of differing dietary pools such as bacteria versus algae (Rieley et al. 1995). The stable isotopic composition of whole tissues provides an integrated measure of the relative contributions of isotopically distinct dietary pools to an organism (Van Dover and Fry 1994) – information complementary, although not necessarily identical, to that of the molecular abundance data. In addition, a combination of the isotopic and molecular abundance approaches by measurement of the stable carbon isotopic compositions of individual lipids extends the information available, allowing for further confidence in inferring dietary sources (Rieley et al. 1995). Therefore, the aim of the present study has been to utilise this combined approach with phospholipid (polar lipid) fatty acids, in order to gain an insight into the trophic ecology of *Rimicaris exoculata* at the TAG and Snake Pit sites along the Mid-Atlantic Ridge. Discussion of the stable isotopic composition of whole tissues and molecular biology of *R. exoculata* are provided in a complementary study by Polz et al. (1999).

## Materials and methods

Samples of alvinocaridid shrimp, *Rimicaris exoculata* were collected during June 1993 by the submersible D.S.V. "Alvin". Three individual mature shrimp were dissected, and the abdominal muscle removed R16, R19 and R34. R16 and R19 were large individuals from the TAG site; R34, also a large individual, was collected from the Moose vent at the Snakepit site. Further large shrimp were selected, and within 1 h upon arrival on board ship the scaphognathites (gill bailers) were removed and scraped with a scalpel to separate the thick masses of epibiotic bacteria from the chitinous cuticle. Scrapings from > 5 individuals were combined prior to deep-freeze storage and analysis. Samples of the metal sulphide deposits around which *R. exoculata* were clustered were also collected. The outer surfaces of these pieces of solid polymetal sulphide mounds and black-smoker chimneys were scraped soon after collection, and combined prior to deep-freeze storage and analysis. Full experimental details of sample workup and analytical procedures are described by Rieley et al. (1995); in brief, samples were freeze-dried and solvent-extracted (Bligh and Dyer 1959). Lipid classes were separated upon silicic acid columns; polar lipid (phospholipid) fatty acids were converted to methyl esters by mild alkaline methanolysis (Guckert et al. 1985). Lipid analyses were undertaken by capillary gas chromatography (GC) and GC–mass spectrometry (GC–MS). Compound specific isotopic analyses were carried out by GC–combustion–isotope ratio–mass spectrometry (GC–C–IRMS) under previously described conditions (Rieley et al. 1995 and references cited therein). In the present study, coelution problems prevented satisfactory  $^{13}\text{C}$  measurement of several important fatty acids such as 18:1(*n*-7) and 20:5(*n*-3) in some of the samples. Under the high loading needed for the GC–IRMS system,

adequate resolution depended on the relative proportions of adjacent peaks, a situation which varied considerably from one type of sample to another. Additionally, minor components were also difficult to measure reliably. Fatty acid nomenclature used in this work follows current International Union of Pure and Applied Chemistry (IUPAC-IUB) conventions.

## Results and discussion

In the deep-sea hydrothermal vent system under study, there are likely to be three main sources of dietary carbon available to *Rimicaris exoculata*: (1) from their epibionts, (2) from the bacteria associated with the sulphides these organisms ingest, and (3) from detritus from the oceanic photic zone. The relative abundances of fatty acids are given in Table 1 and summarised in Fig. 1. All samples had high abundances of saturated and monounsaturated fatty acids and of fatty acids unsaturated seven carbons from the terminal methyl (*n*-7 fatty acids). The bacterial samples were characterised by especially high abundances of (*n*-7) fatty acids (> 75% of total fatty acids; Table 1; Fig. 1), which are common bacterial marker lipids (Zhukova et al. 1992). Correspondingly high abundances of (*n*-7) fatty acids in the *R. exoculata* samples (> 40% of the total) indicate a diet rich in bacteria (Zhukova et al. 1992; Rieley et al. 1995) which, together with relatively low abundances of polyunsaturated fatty acids, PUFAs (< 36% of total), differentiate *R. exoculata* from coastal and estuarine shrimp reliant upon a phytoplankton-based diet (e.g. *Neomysis integer*: Bradshaw et al. 1990; *Artemia* sp.: Webster and Lovell 1991; present Fig. 1).

Comparison of the epibiont and sulphide fatty acid profiles reveals a close similarity (Table 1; Fig. 1), reflecting the results of Polz and Cavanaugh (1995) who used 16-sRNA techniques to demonstrate that the most abundant bacteria in the sulphides are genetically identical to the epibiotic bacteria of *Rimicaris exoculata*. The predominant difference between the two profiles is in the presence of low abundances of polyunsaturated fatty acids in the epibiont sample (Table 1). However, it is possible that these acids came from a small amount of host tissues being incorporated into the epibiont samples during scraping of the gill bailers.

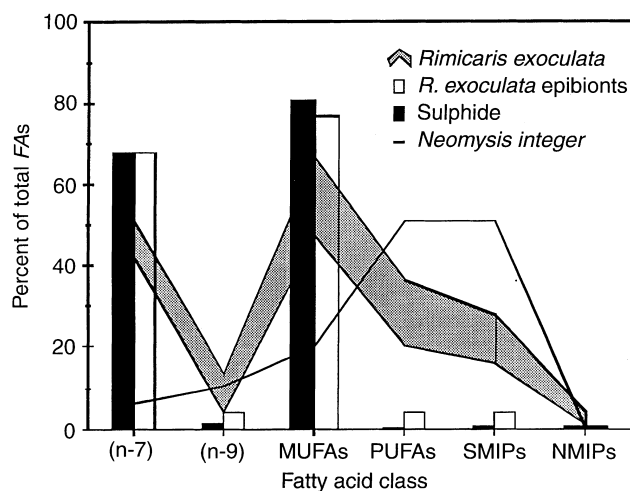
Isotopic compositions ( $\delta^{13}\text{C}$ ) of total tissue organic carbon give an integrated measure of all carbon sources and little information as to specific sources of carbon. Isotopic analyses of individual lipids can unlock this information using knowledge of biosynthetic pathways (Rieley et al. 1995, 1997). The current study analysed polar lipid (phospholipid) fatty acids, which are constituents of cell membranes, since these are the most abundant lipid class in most organisms and are generally more biosynthetically controlled than storage lipids. Certain specific fatty acids can have distinct sources: for example, palmitic acid (16:0) is common to virtually all eubacteria and eukaryotes, whereas 18:1(*n*-7) is biosynthesised de novo by eubacteria. This 18:1 acid can, of course, also be formed by chain elongation of the commonly available 16:1(*n* = 7). In addition, polar

**Table 1** *Rimicaris exoculata*. Relative concentrations of polar lipid fatty acids (FAs, % of total) in shrimp from Mid-Atlantic Ridge [R16, R19, R34 shrimp abdominal muscle tissue from individual shrimp; Epibionts scrapings of bacteria from shrimp carapaces (pooled from > 5 individuals); Sulphide sulphide samples from regions of shrimp clusters]

FAs	Epibionts	Sulphide	R16	R19	R34
14:0	6.5	1.3	0.1	0.1	0.1
15:0	0.1	0.3	0	0	0
16:0	9.2	11	8.6	10.7	9.4
17:0	0.1	0.2	0.1	0.2	0.1
18:0	1.8	0.9	3.8	5.8	5.6
20:0	0.3	0.1	0.2	0	0.2
22:0	0	0	0.1	0	0
i15:0	0.2	1.9	0	0	0
a15:0	0.4	2.2	0	0	0
i16:0	0.1	0.2	0	0	0
i17:0	0.1	0.2	0.1	0.1	0.2
cy17:0	0.2	0.9	0	0	0
cy19:0	0.1	0.3	0	0	0
14:1(n-5)	0.2	0	0	0	0
14:1(n-7)	3.2	0	0	0	0
16:1(n-5)	5.2	10.5	0.4	0.3	0.2
16:1(n-7)	40.8	35.4	10.5	6.1	9.3
tr-16:1(n-7)	0	4.8	0.1	0.1	0.1
16:1(n-9)	0	0.7	0	0	0
16:1	0	0	0	0	0
18:1(n-5)	0.4	0.6	1.1	0.8	0.8
17:1(n-8)	0.2	0.3	0.2	0.1	0.3
18:1(n-7)	21.3	24.2	38.4	34.3	32.5
tr-18:1(n-7)	0.5	3.1	0	0	0
18:1(n-9)	3.6	0.4	13.1	3.6	9.7
19:1(n-12)	0	0	0.1	0	0.2
19:1(n-8)	0.2	0	0.3	0.1	0.2
20:1(n-7)	0.5	0.2	1.1	1	0.9
20:1(n-9)	0.1	0	0.3	0.2	0.3
21:1	0	0	0	0	0
21:1	0.1	0	0.1	0.1	0.1
22:1(n-7)	0.4	0	0.9	0.4	0.8
a17:1(n-8)	0.1	0.5	0	0	0
br17:1	0	0	0.1	0	0.1
br17:1	0	0	0	0	0
18:2	0.2	0	0	0	0.1
20:2Δ5,11	0.2	0	0.2	0.1	0.4
20:2Δ5,13	1	0	4	0.9	3.8
18:3(n-3)	0.6	0	0	0	0.2
18:4(n-3)	0	0	0	0	0
20:4(n-6)	0.7	0	2.4	3.1	1.5
22:4(n-6)	0	0	0.1	0	0.1
20:5(n-3)	1	0	9.9	14.2	10.9
22:5(n-3)	0	0	0.6	0.5	0.7
22:6(n-3)	0.5	0	3.2	17.4	11.3

lipids are degraded rapidly after cell death (Guckert et al. 1985); therefore, in the case of the metal sulphides, the isotopic composition of the polar lipid fatty acids extracted from them represent that of in situ, living bacteria. Sterols were also analysed where present, since this class of compound also has important biosynthetic characteristics; for example, bacteria generally cannot biosynthesise sterols and neither can crustaceans (Kerr and Baker 1991). The isotopic composition of individual fatty acids and of shrimp cholesterol are given in Table 2 and summarised in Fig. 2.

The bacterial populations of *Rimicaris exoculata* epibionts and those of the sulphides are largely in-



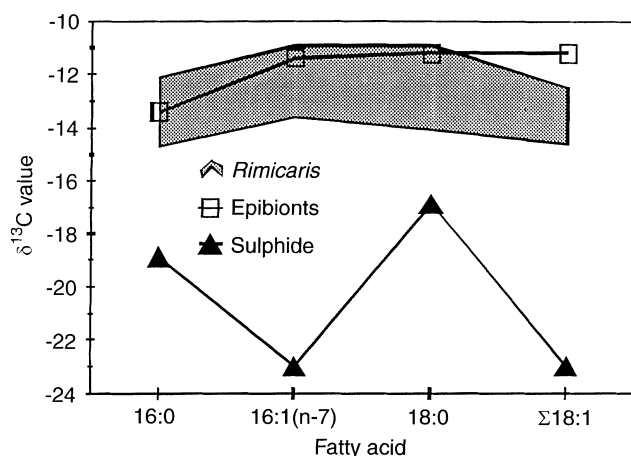
**Fig. 1** *Rimicaris exoculata*. Phospholipid fatty acids isolated from Mid-Atlantic Ridge samples, as percent of total fatty acids (FAs) identified (as their methyl ester derivatives). *R. exoculata* values are range of three separate individuals. *Neomysis integer* data from Bradshaw et al. (1980) are shown for comparison [*n-7*, *n-9* fatty acids related by position of terminal unsaturation; *MUFAs* monounsaturated fatty acids; *PUFAs* polyunsaturated fatty acids; *SMIPs* single methylene-interrupted *PUFAs*; *NMIPs* non methylene-interrupted *PUFAs* (cf. Rieley et al. 1995)]

distiguishable by both genetic analyses (Polz and Cavanaugh 1995; Polz et al. 1999) and by fatty acid abundances (Table 1; Fig. 1). However, the  $\delta^{13}\text{C}$  values obtained indicate that the isotopic compositions of the individual fatty acids of the sulphide bacteria (ranging from  $-17$  to  $-23\text{‰}$ ) are more  $^{13}\text{C}$ -depleted than those (ranging from  $-11$  to  $-13\text{‰}$ ) of the *R. exoculata* epibionts (Table 2). Such differences are also reflected in the total organic carbon  $\delta^{13}\text{C}$  values (Polz et al. 1999). Since the epibiotic and sulphidic bacterial populations are genetically indistinguishable, the question arises as to what causes the isotopic difference observed between the two populations. There are several possibilities: (1) a difference in the  $^{13}\text{C}$ -composition of source carbon for the bacteria, (2) carbon limitation on the bacterial population within the shrimp carapace, and (3) differences between the (kinetic isotope) discrimination against  $^{13}\text{C}$  of the RuBisCO enzyme of the two bacterial populations (Robinson and Cavanaugh 1995). Since *R. exoculata* live mainly within the zone (a few centimetres thick) of the vent fluids immediately surrounding the sulphides, the isotopic composition of source  $\text{CO}_2$  should be very similar in both cases. There may be some microenvironmental differences in the isotopic composition of  $\text{CO}_2$  (e.g. due to temperature); however, such differences are likely to be minor and would not explain the significantly heavier  $^{13}\text{C}$  composition of the epibiotic bacteria.

The difference in the isotopic compositions of the two bacterial populations is most likely due to the expression of different forms of RuBisCO, as has been reported for other hydrothermal symbionts (Robinson and Cavanaugh 1995). The two forms, I and II, discriminate

**Table 2** *Rimicaris exoculata*. Stable carbon isotopic compositions (‰ Pee Dee Belemnite) of individual lipids from Mid-Atlantic Ridge [Values in parentheses standard deviations of replicate capillary gas chromatography–combustion–isotope ratio–mass spectrometry (GC/C/IRMS) runs; further details as in legend to Table 1]

Lipid	Epibionts	Sulphide	R16	R19	R34
14:0	-12.5(0.7)				
16:0	-13.4(0.3)	-18.9(0.4)	-12.2(0.3)	-14.8(0.4)	-14.0(0.3)
18:0	-11.2(0.2)	-16.9(0.8)	-11.0(0.7)	-14.2(0.3)	-11.9(0.5)
i15:0		-22.9(0.5)			
a15:0		-21.3(0.5)			
14:1(n-7)	-12.4(0.4)				
16:1(n-7)	-11.4(0.5)	-23.0(0.7)	-10.9(0.4)	-11.5(0.5)	-13.7(0.3)
18:1(n-9)	-13.3(0.4)				
18:1(n-7)	-11.8(0.5)	-22.8(0.8)			
18:1(n-5)			-13.9(0.8)	-13.0(0.1)	-14.9(0.2)
Σ18:1	-11.2(0.2)		-12.6(0.1)	-14.7(0.7)	-13.7(0.5)
20:1(n-9)			-10.4(0.4)		-12.1(0.3)
20:2Δ5,13			-12.1(0.3)		-15.0(0.3)
20:4(n-6)				-15.6(0.4)	
22:6(n-3)			-13.1(0.4)	-15.7(0.2)	-14.1(0.2)
cholesterol			-26.0(0.3)		-26.1(0.4)



**Fig. 2** *Rimicaris exoculata*.  $\delta^{13}\text{C}$  values (‰) of individual phospholipid fatty acids isolated from samples collected from Mid-Atlantic Ridge. Shrimp data are for muscle tissue (range of three separate individuals); epibionts were scraped from carapaces of fully grown shrimp, and sulphides were collected from areas of large shrimp clusters

against  $^{13}\text{C}$  to a different extent, Form I discriminating the most. Carbon limitation may also contribute to the  $^{13}\text{C}$ -depletion of the epibionts, especially of the larger *Rimicaris exoculata*, because of the close packing of the microbial population and sulphides in the shrimp carapace.

The  $\delta^{13}\text{C}$  values for *Rimicaris exoculata* fatty acids range from  $-10$  to  $-16$ ‰ compared to bulk-tissue values averaging  $-12$ ‰ (Polz et al. 1999). Comparison of the fatty acid  $\delta^{13}\text{C}$  values of *R. exoculata* muscle tissue, the epibionts, and the sulphides demonstrates a very close relationship between the former two. This relationship, together with the fact that bulk organic carbon from the oceanic photic zone has a  $\delta^{13}\text{C}$  value of around  $-22$ ‰ (Abelson and Hoering 1961), clearly indicates that the main source of dietary carbon for mature *R. exoculata* is from epibionts associated within their carapace and mouthparts. A relatively small dietary contribution direct from the bacterial population of the sulphide

mounds may explain the observation that *R. exoculata* tend to have fatty acid  $\delta^{13}\text{C}$  values which are 1 to 2‰ more negative than those of their epibionts. However, the bacterial population actually associated with the metal sulphides is quite small (Polz and Cavanaugh 1995), and either carbon limitation, as mentioned above, or a contribution from gut microflora could also explain such a difference (Polz et al. 1999).

In contrast to the fatty acids, which reveal a close isotopic relationship between epibiont and *Rimicaris exoculata* polar lipids, cholesterol isolated from the shrimp tissues is much more  $^{13}\text{C}$ -depleted (averaging  $-26$ ‰; Table 2) than all the other lipid fractions examined. As expected, no sterols were observed in the bacterial samples, and since crustaceans cannot biosynthesise sterols (Kerr and Baker 1991), then detritus derived ultimately from the oceanic photic zone is the principal likely source and, indeed, the isotopic composition of *R. exoculata* cholesterol is close to that expected for lipids from marine plankton (Abelson and Hoering 1961). Such an input of carbon to this site from the photic zone of the ocean, albeit in relatively minor amounts of this key biochemical nutrient, would accord with the claim that, although predominantly fuelled by primary production of chemoautotrophic bacteria, deep-sea hydrothermal vents have a small but important input of photosynthetically fixed carbon (Dixon et al. 1995; Rieley et al. 1995). Vent biota and deep-sea benthos generally presumably avidly conserve and recycle essential fatty acids and sterols reaching them, since such compounds are a scarce benthic resource. A great deal of further isotopic information on these vent foodwebs undoubtedly resides in the compound-specific  $\delta^{13}\text{C}$  values of the full range of fatty acids present in the various fractions. However, enhanced separation procedures capable of resolving peaks due to coeluting isomers and other closely-related compounds will be needed if the full scope of this approach is to be realised. For example, in the present study, coelution problems prevented satisfactory  $^{13}\text{C}$  measurement of several important fatty acids such as 18:1(n-7) and 20:5(n-3), in some of the samples. This was unfortunate (although

unavoidable with the available methodology), since these acids can be expected to carry highly specific information about their origin in their individual  $\delta^{13}\text{C}$  values. Thus, more complete compound-specific data should help address the present ambiguity in the origins of PUFAs. Like cholesterol, PUFAs must largely be acquired by the shrimp, presumably also ultimately from the photic zone; but our limited data do not discriminate them isotopically from the other fatty acids. Analysis of water-column particulates at this site from the photic, intermediate, and benthic zones would clarify this.

The abundance patterns and stable isotopic compositions of fatty acids extracted from samples of the vent shrimp *Rimicaris exoculata* and metal sulphides from the Mid Atlantic Ridge provide strong evidence that *R. exoculata* from the TAG and Snake Pit hydrothermal fields gain most of their carbon from the epibiotic bacteria living within their carapaces. Differences in the isotopic discrimination of two forms of the enzyme RuBisCO (Robinson and Cavanaugh 1995: Form II in the epibiotic bacteria and Form I in the bacteria growing in the mineral sulphide deposits) may explain the enriched  $^{13}\text{C}$ -composition of *R. exoculata* observed in this and other studies (Van Dover et al. 1988; Polz et al. 1999). Carbon limitation experienced by the epibiont populations within the larger shrimps may also be a significant factor.

The isotopic composition of cholesterol extracted from *Rimicaris exoculata* indicates that this lipid may derive ultimately from the oceanic photic zone.

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