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Polar lipid fatty acids as indicators of trophic associations in a deep-sea vent system community

Colaço, Ana[1*]; Desbruyères, Daniel[2]; Guezennec, Jean[3]

1 IMAR – Department of Oceanography and Fisheries University of the Azores, Cais de Sta. Cruz, Horta (Azores), Portugal
2 DEEP/Laboratoire Environnement Profond, Centre de Brest de l'IFREMER, Plouzane Cedex, France
3 Département Biotechnologies Marines, Centre de Brest de l'IFREMER, Plouzane Cedex, France

*: Corresponding author : acolaco@notes.horta.uac.pt

Abstract:

The polar lipid fatty acid (PLFA) profiles of invertebrates living in chemosynthetic communities can indicate the degree to which these animals depend on specific types of bacteria. To identify the nutritional sources of various species from deep-sea hydrothermal vents of the Mid-Atlantic Ridge, a Principal Component Analysis was performed using individual PLFA profiles as descriptors. Two associations representing different feeding groups were identified: (i) mussels, commensal polychaetes and gastropods, (ii) shrimps and crabs. The first association relies more on sulphide-oxidizing bacteria, while the second one has more anaerobic sulphate-reducing bacteria biomarkers. Other small invertebrates reveal different diets. The polychaete Amathys lutzi shows the most diversified bacterial diet, with fatty acid biomarkers from both S-oxidizing and S-reducing bacteria.

Keywords: fatty acids, hydrothermal vents, biomarkers, Mid-Atlantic Ridge

Abstract:

The polar lipid fatty acid (PLFA) profiles of invertebrates living in chemosynthetic communities can indicate the degree to which these animals depend on specific types of bacteria.

To identify the nutritional sources of various species from deep-sea hydrothermal vents of the Mid-Atlantic Ridge, a Principal Component Analysis was performed using individual PLFA profiles as descriptors. Two associations representing different feeding groups were identified: (i) mussels, commensal polychaetes and gastropods, (ii) shrimps and crabs. The first association relies more on sulphide-oxidizing bacteria, while the second one has more anaerobic sulphate-reducing bacteria (SRB) biomarkers. Other small invertebrates reveal different diets. The polychaete *Amathys lutzi* shows the most diversified bacterial diet, with FA biomarkers from both S-oxidizing and SRB bacteria.

Problem

It is widely known that fatty acid analyses (FA) can be good indicators of specific microorganisms, since different groups of bacteria may have different FA

compositions (Lechevalier, 1977; Goodfellow and Minnikin, 1985). Hence, FAs have been used in classical enumeration procedures to reveal and estimate which microorganisms are associated with biofilms, soils and sediments (Guezennec et al., 1998) (table 1).

Polar lipid fatty acids (PLFA) are good biomarkers for disclosing and describing trophic relations between vent community animals, as well as for confirming the importance of bacteria in the nutrition of organisms endemic to hydrothermal vents. Highly unsaturated fatty acids (HUFA) composed of long carbon chains (e.g., C20:4ω6, C20:5ω3 or C22:6ω3) can be indicative of phytoplankton (Chuecas & Riley, 1969), although some bacteria can also synthesize them (Nichols 2003). These HUFA are characteristic of all heterotrophic marine invertebrates and are usually present in high concentrations in the tissue of these organisms (Sargent et al., 1990). Therefore, very low levels of HUFA in the hydrothermal vent communities suggest a trophic web that is not primarily based on phytoplankton. Likewise PLFA in invertebrates living in communities where the food chain is based on microorganisms can indicate the degree to which these animals feed on bacteria and suggest which bacteria they rely on, since bacteria-specific PLFA can be used as biomarkers (Guezennec, 1995).

It has been proposed that animals whose diets predominantly consist of bacteria, i.e., diets rich in 16:0, 16:1(n-7), and 18:1(n-7) fatty acids, while being relatively deficient in (n-3) PUFAs, produce Non-methylene interrupted dienoic (NMID) fatty acids from monenoic fatty acids (Ackman and Hooper, 1973) which effectively substitute for the low levels of (n-3) PUFAs (Pond et al, 2002). This FA have been observed in symbiotic organisms and some molluscs (Conway & Capuzzo, 1991).

1 Several authors have studied FA composition and compound-specific stable isotopes
2 of Mid Atlantic Ridge hydrothermal vent invertebrate species (Pond et al., 1997a,
3 1997b, 1998; Rieley et al., 1999; and Allen et al., 2001). Although these studies have
4 contributed greatly to the knowledge of the feeding strategies of individual species,
5 they did not establish trophic links between species and their respective predators. A
6 special case is the dominant vent shrimp at the deeper Mid-Atlantic Ridge
7 hydrothermal vent sites *Rimicaris exoculata*. It is the only species that actively seeks
8 the active facies from the hydrothermal structures and several hypotheses developed
9 the importance of bacteria in the nutrition of these shrimps. In this study we ask: (1)
10 What is the relationship between the lipid profiles of the various species? (2) Can
11 trophic relationships be established from PLFA profiles? (3) What are the feeding
12 strategies of *Rimicaris exoculata*?
13
14 **Material and Methods:**
15 **Animal collection**
16 Samples were taken during three deep-sea hydrothermal vent cruises along the Mid-
17 Atlantic Ridge (MAR). MAR/97, in 1997, sampled all of the known hydrothermal
18 vent fields along the MAR at that time. (Menez Gwen 37,51ºN 840m; Lucky Strike
19 37,18ºN 1700m; Rainbow 36,13ºN 2300m; Broken Spur 29,10ºN 3000m; TAG
20 26,08ºN 3600m; Snake Pit 23,22ºN 3400m, and Logatchev 14,45ºN 2900m); two
21 other cruises (MARVEL in 1997 & PICO in 1998) sampled the Azores Triple
22 Junction hydrothermal vent fields (Menez Gwen; Lucky Strike; Rainbow). Animals
23 were collected using the submersible's packman scoop, or by using a slurp gun.
24 *Mirocaris fortunata, Chorocaris chacei,* and *Rimicaris exoculata* shrimps;
25 *Bathymodiolus* spp mussels; the *Segonzacia mesatlantica* crab; and the

1 *Phymorhynchus moskalei* whelk were dissected immediately upon arrival on board in
2 order to retrieve the digestive gland. The mouth parts were retrieved only from the *R.*
3 *exoculata* shrimp. The remaining specimens collected were not dissected due to their
4 reduced size. Samples were subsequently deep-frozen (-80ºC) on board and freeze-
5 dried at the shore laboratory. Whole animal PLFA profiles were determined in the
6 polychaete *Amathys lutzi* (n=5), the polychaete *Branchipolynoe seepensis* (n=12)
7 commensal with mussels, the pycnogonid *Sericosura* sp. (n=2), the amphipods (n=2)
8 (unknown species) and the shrimp *Alvinocaris markensis* (n=1).
9 PLFA profiles were also determined for the digestive glands of the mussels (n=26)
10 (*Bathymodiolus azoricus, B. puteoserpentis and Bathymodiolus spp.*), crab (n=15)
11 (*Segonzacia mesatlantica*), whelk (n=6) (*Phymorhynchus* sp.), and shrimps (*R.*
12 *exoculata* (n=13), *C. chacei* (n=2) and *M. fortunata* (n=6)) , along with *R. exoculata*
13 mouthparts (n=4).
14 **Lipid analysis**
15 Lipids were extracted using a modified version of the Bligh and Dyer method (Bligh
16 and Dyer, 1959; White et al., 1979). Total extractable lipids were fractionated by
17 silicic acid column chromatography. The PLFA esters were methylated by acid
18 methanolysis of the polar lipid fractions. Quantification was based on comparing
19 peak areas to an internal injection standard (19:0). Gas chromatography was carried
20 out using a HRGC 5160 Megaseries (Carlo-Elba) equipped with an injection system
21 *on column*, and with a flame ionisation detector (FID), connected with an integrator
22 SP42070 (Spectra Physics) D-2500 (Merck). Chromatography was carried out using
23 a non polar column CP-Sil-5 CB® (Chrompack).
24 **FA STRUCTURAL VERIFICATION**

Two-derivatization methods (DMDS and D-MOX) were used to identify the mono and polyunsaturated double bond position (Guezennec, 1986; Nichols et al., 1986; Yu et al., 1989; Fay and Richli, 1991). FA identification was by GC/MS. GC-MS was carried out using a HRGC 5160 Megaseries (Carlo-Elba) chromatograph equipped with a NERMAG R 10 x 10 quadripolar mass spectrometer.

STATISTICS

To identify associations between the different species, principal component analysis (PCA) was performed using individual PLFA profiles as descriptors for the different individuals. The matrix was standardized by dividing each row by the standard deviation and subtracting off the mean of each row.

We hypothesize that if the species fall within the same group, it signifies a strong association among the species. They either eat the same food source or feed on each other. The higher trophic level (predation or omnivory) is established when two species are associated and one presents lower amounts of bacterial origin fatty acid.

Due to the low number of samples, no statistical analysis was performed to compare the PLFA profiles of the digestive glands and mouth parts of the shrimp *R. exoculata.* Graphs are presented comparing the PLFA profiles.

RESULTS

RELATIONSHIPS BETWEEN TAXONOMIC GROUPS

Table 2 summarizes the percentage of each PLFA for each species or taxonomic group. Several PLFA profiles were assessed for each taxonomic group; however, no specific PLFA profile could be established for a species or taxonomic group, although the percentages obtained in some cases merit attention.

1 The pycnogonid *Sericosura* sp. and the *M. fortunata* and *C. chacei* shrimps have a
2 high percentage of C18:0. For mussels and the polychaete *A. lutzi*, C16:0 is present at
3 more than 20 percent. The *R. exoculata* and *M. fortunata* shrimps show large
4 amounts of C14:0.
5 The percentage of monounsaturated fatty acid (MUFA) varied considerably between
6 all the groups. The shrimp *R. exoculata* was the only species that possessed
7 C14:1ω7. The fatty acids C16:1ω9 and C16:1ω7 were present at levels of more than
8 8% in all the groups except for the pycnogonid *Sericosura* sp. and the whelk
9 *Phymorhynchus* sp. The highest percentages of C18:1ω13+C18:1ω9 were found in
10 the amphipods, the shrimp *A. markensis*, and the crab *S. mesatlantica*. C18:1ω7 was
11 important in all taxonomic groups except for the mussels. The whelk *Phymorhynchus*
12 sp. had noticeable amounts of C20:1ω13 and C20:1ω9 as did the commensal worm
13 (*B. seepensis*) and the mussels. C20:1ω7 is present in similar amounts in the
14 polychaete *A. lutzi*, commensal worm, mussel and whelk, but was present at low
15 levels in the other taxonomic groups. C21:1ω9 was found exclusively in the
16 polychaete *A. lutzi.*
17 The highest percentages of branched PLFA were seen in the polychaete *A. lutzi* and
18 the shrimps *R. exoculata* and *M. fortunata*. However, iC19:0 was an exception, with
19 highest percentages found in the mussel, followed by the shrimp *M. fortunata* and the
20 amphipod. Polyunsaturated fatty acids (PUFA) were present in different quantities in
21 all the taxonomic groups sampled. Nearly all the animals contained nonmethylene-
22 interrupted dienoic fatty acids (NMID) from the Δ5-desaturation pathway. A higher
23 percentage of the PUFA C18:3ω (9,12,15) was present in mussels and amphipods
24 than in the other taxonomic groups (<1%). High proportions of the NMID
25 C20:2ω(7,15) were found in mussels, the shrimp *R. exoculata* and the whelk

Phymorhynchus sp. The mussel and commensal worm had relatively high percentages of the NMID C20:2ω(6,15), as did the whelk and the amphipods. The percentage of NMID C20:2ω(8,15) was greater than 1% in the polychaete *A. lutzi*, in the commensal worm, mussels, whelk, amphipod and in the shrimp *M. fortunata*.

The PLFA profiles of the digestive gland or the entire animal were used as descriptors to perform the principle component analysis (PCA) (Fig. 1). The first component differentiates the mussels *Bathymodiolus* spp, the *Phymorhynchus* sp. whelk and the commensal worm *B. seepensis*. The shrimps and crab are grouped separately, and at the center of the axes other non-dominant invertebrates, such as the polychaete *Amathys lutzi*, the pycnogonid *Sericosura* sp., and amphipod, are identified. The second component differentiates the mussel, shrimps and part of the crab from the commensal worm, the whelk *Phymorhynchus* sp., the polychaete *A. lutzi* and the remaining crabs.

PARTICULAR CASES:

Shrimp *Rimicaris exoculata*

For a better understanding of the *R. exoculata* food source, the PLFA profiles of mouthparts and digestive gland were compared (Figures 2). The profiles of these tissues in *R. exoculata* individuals, three from Rainbow hydrothermal field and the other from Lucky Strike hydrothermal field, overlap. With the exception of some NMID, both individuals exhibited similar PLFA profiles. Generally the mouthparts presented higher quantities of the same MUFAs and branched fatty acids than the digestive gland.

1 **Mussels (*Bathymodiolus azoricus, B. puteoserpentis, Bathymodiolus*
2 **spp.)**

3 Despite the existence of S-oxidizing and methanotrophic endosymbiotic bacteria in
4 mussel gill tissue (Fiala-Médioni et al, 2002), no typical methanotrophic bacteria
5 biomarkers were found. However, the large percentage of iC19:0 and C18:1ω13 +
6 C18:1ω9 is certainly related to the presence of endosymbiotic bacteria.
7

8 ## DISCUSSION

9 The presence of bacteria-specific biomarkers such as iC15:0 and iC17:1ω7, as well
10 as large quantities of the 1ω7 series, low levels of PUFA, the absence of HUFA
11 (notably the absence of C20:5ω-3 and C22: 6ω-3, which are phytoplankton
12 biomarkers) and the presence of NMID all indicate that the vent communities rely
13 almost entirely on bacteria. Those species that present more branched fatty acids
14 from the ω7 series rely more on S-reducing bacteria, while the ones with more
15 MUFA from the ω7 series and more NMID from this series rely more on S-oxidizing
16 bacteria.
17

18 ### RELATIONSHIPS BETWEEN SPECIES

19 The most striking result of the PCA analyses was the observation of two different
20 groups. Despite the fact that no species presented a specific PLFA profile, organism
21 associations were found. The two associations: mussel-commensal worm-whelk and
22 shrimps-crab have different PLFA indicative of different bacteria in their diets. The
23 mussel-commensal worm-whelk association relies more on S-oxidizing bacteria,
24 while the crab-shrimp association has more S-reducing bacteria (SRB) anaerobic
25 biomarkers. This indicates that the latter group of animals apparently lives in

1 conditions closer to anoxia than the other, which is congruent with the
2 microdistribution of these organisms (Desbruyères et al., 2001). The large central
3 group represents omnivorous species that feed on bacteria in addition to other types
4 of food (e.g., *C.* chacei). The individual fatty acids do not differentiate groups; but
5 the percentage and composition of PUFA are responsible for the observed
6 distribution.
7 These two associations (mussel-commensal worm-whelk and shrimps-crab) were
8 also demonstrated by stable isotope analysis, with the first association presenting
9 very depleted $\delta^{13}C$ (around -30‰), and the second presenting less depleted $\delta^{13}C$
10 (around -11‰) values (Colaço et al., 2002). .
11 The individuals at the center of the PCA axes have a more diversified bacterial diet,
12 with several different biomarkers from S-oxidizing and SRB bacteria. The
13 association of mussels and *Phymorhynchus* sp. was also observed through stable
14 isotope analysis (Fisher et al., 1994; Colaço et al., 2002) as was the mussel-
15 commensal worm coupling (Colaço et al., 2002).
16 The fatty acid composition of *M. fortunata* fell midway between that of the other
17 shrimp species (*R. exoculata, C. chacei*, *A. markensis*). *R. exoculata* is considered to
18 be bactivorous, and *C. chacei* and *A. markensis* to be carnivorous or scavengers. The
19 PLFA profile of *M. fortunata* indicates that this species is omnivorous, feeding not
20 only on other animals (carnivorous) but also on S-oxidizing bacteria (bactivorous), as
21 indicated by the presence of branched fatty acids, monounsaturated ω7 fatty acids
22 and more NMID (c. f. Table 2). However, according to our definition, this species
23 might also be a secondary consumer, eating animals that are eating the bacteria, as
24 was indicated by Colaço et al., 2002. The crab *S. mesatlantica* shows a similar
25 pattern to that of *M. fortunata*, despite possessing fewer branched fatty acids.

According to Colaço et al., 2002, the crab's trophic position is not that of omnivore, but rather predator, feeding on bactivorous animals like amphipods and other mixotrophs like shrimps. If this is the case, the bacterial FA present in these animals is from the prey food source and not from bacteriophagy.

The polychaete *A. lutzi* presented a characteristic fatty acid profile, with the higher amounts of branched fatty acids indicative of a diversified bacterial diet. Its mobility allows it to feed on different food sources at the hydrothermal vents.

Detailed analyses of the fatty acid profiles shows an absence of HUFA (C20:5ω3 and C22:6ω3) in all the taxonomic groups studied, suggesting that if the vent fauna relies on photosynthetically produced material, it is as transformed material like nutrients or polysaccharide molecules, rather than lipids. The low amounts of PUFA also support this idea, especially in light the absence of C20:4ω6.

The presence of phyto-biomarkers at some hydrothermal vent fields (e.g. 13ºN) and their absence at the Galapagos field was tentatively related to the mussel's condition. The condition parameter was the loss of symbionts (Ben-Mlih et al., 1992). This situation is not surprising, since the mussels did not lose their filter-feeding capacity, and in the absence of symbionts, phytodetritus-derived particles would be one of the available energy sources. Mussel symbionts disappear when the mussel environment loses the symbionts' energy source (Dando, pers. com.)

The PUFA present in the PLFA lipids of the studied species are due to two factors, the desaturation possible in all aerobic Δ9 organisms, and the desaturation specific to certain Δ5 marine invertebrates. The presence of high proportions of NMID from C18:1ω7 is a clear indicator that the species rely on chemoautotrophic bacteria, as has already been indicated by several authors (Zhukova, 1991; Pond et al., 1997b; Pranal et al., 1997).

PARTICULAR CASES

Shrimps *Rimicaris exoculata*

In the deep-sea hydrothermal vent fields at MAR, the three main sources of dietary carbon that are available to the shrimp *R. exoculata* are: (i) bacteria fixed on the mouthparts of this species; (ii) bacteria associated with the minerals that these organisms ingest from the sulphide chimneys, and (iii) detritus from the oceanic photic zone. The absence of HUFA, the low levels of PUFA with the exception of the NMID, makes the third hypothesis rather improbable, consistant with the results of Rieley et al. (1999). The lipid profile of this species is also different from deep-sea benthic shrimps (*Nematocarcinus gracilis*) that rely on phototrophically derived organic matter (Allen, 1998). However, and according to Pond et al., (1997a), juvenile *Rimicaris exoculata* rely on material from the photic zone of the ocean. Allen et al. (2001) also showed that in this species, adults and juveniles exhibit different partitioning of lipids in tissues, with an increased proportion of bacterial-derived organic matter in adults compared to that of juveniles.

Comparison of mouthparts and digestive gland PLFA profiles revealed a close similarity, with the digestive gland having more NMID. When looking at the monounsaturated fatty acids that might underlie the origin of these NMID, the FA profiles of the mouthpart and the digestive gland are very similar. Earlier studies showed that the bacteria from the mouthparts (epibionts) and bacteria from sulphides are closely related (Rieley et al., 1999). However, by means of a specific compound stable isotope analysis, it was shown that the epibiont bacteria have a $\delta^{13}C$ signal similar to that of the shrimp, while the sulphide bacteria are much more ^{13}C depleted. Considering these factors, and from the results presented here, we conclude that this species relies on bacteria present in its mouthparts.

Mussels (*Bathymodiolus azoricus, B. puteoserpentis, Bathymodiolus* spp.).

Analyses of fatty acids as biomarkers often allow for an evaluation of host-symbiont energy relationships and carbon sources (Jahnke et al., 1995; Pranal et al., 1997). Despite the fact that the mussels host S-oxidizing and methanotrophic bacteria (Fiala- Medioni et al., 2002), no methanotrophic biomarker was observed on the mussel PLFA profiles. This result contradicts Pond et al. (1998), in which these biomarkers were found in mussels from Lucky Strike and Menez Gwen. Type I Methanotroph biomarkers (C16: 1ω6; and C18:1ω6) were observed in other species but in smaller amounts. The presence of the fatty acid C18:1ω13, a methylotrophic bacteria fatty acid, do not allow the determination to which type of methanotrophic bacteria the mussels rely on, since Methylotrophs are ubiquitous microorganisms that can use C1 compounds (methanol, methylamine, methane, etc.) for growth and just some specialized methylotrophs, are the methanotrophs. Bowman et al. (1991) characterised several methanotrophic bacteria from PLFA profiles and showed that some bacteria did not present the typical biomarkers of the methanotrophic types. Despite the lack of typical methanotrophic PLFA, the presence of other methanotrophic bacteria cannot be excluded. The presence of NMID C20:2ω6, 15 and C20:2ω8,15 in mussels leads us to believe that these fatty acids are the result of a specific biosynthetic pathway based on chain elongation and original Δ5 desaturation of the fatty acids C16:1ω6; C16:1ω8; 18:1ω6 and C18:1ω8 (which are methanotrophic biomarkers). The methanotrophic FA published in Pranal, et al, 1997, were not detected in the mussels. As the deep-sea hydrothermal environment is continually changing, the hypothesis that the mussels have lost methanotroph symbionts and began using other symbionts cannot be ruled out. The presence of S-oxidizing bacteria biomarkers (C16:1ω7; and C18:1ω7; C20:11ω7) and NMID

derived from fatty acids (ex: C18:3ω7,10,13; C20:3ω7,10,15; C20:2ω7,15) and the absence of HUFA are in agreement with results of Pond et al. (1998) and are consistent with other studies on animals hosting S-oxidizing endosymbiotic bacteria or feeding on them (Conway and Cappuzo, 1991; Zhukova, 1991; Zhukova et al., 1992; Fullarton et al., 1995; Pranal et al., 1997)

The presence of PLFA from S-reducing bacteria in mussels proves that they continue to be filter feeders.

Conclusions

Fatty acids from polar lipids can be used to reveal associations among invertebrate species, revealing carbon utilization patterns between species and identifying the organisms that rely on the same type of bacteria. Such associations imply that the species eat the same food types, or that there is a prey-predator relationship. The associations revealed here showed that some species rely more on S-oxidizing bacteria (mussel-commensal-whelk), while the shrimp and crabs rely more on S-reducing bacteria. The PLFA can be used to establish trophic relationships in the vent environment. However to generalize there is a need to validate the hypothesis that prey and predator present similar fatty acids or elongation, and this has only been done for the relationship between the primary producers (bacteria and phytoplankton) and the primary consumers.

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1 Fig.1 PCA performed on the fatty acid profiles of all samples. Component 1 represents
2 16.81% of the variance, and component 2 represents 12.52% of the variance. The
3 eigenvalues are 14.80 and 11.04.
4
5 Fig. 2 PLFA profiles from *Rimicaris exoculata* mouthpart and digestive gland. **A** represents
6 the Rainbow hydrothermal vent field (n=3), **B** represents the Lucky Strike hydrothermal vent
7 field (n=1).
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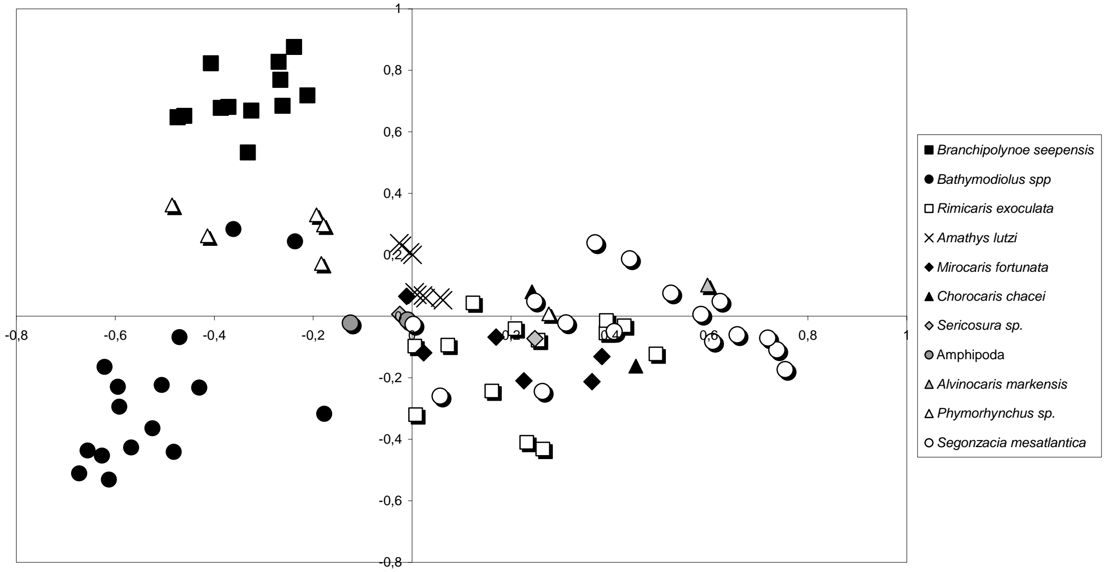

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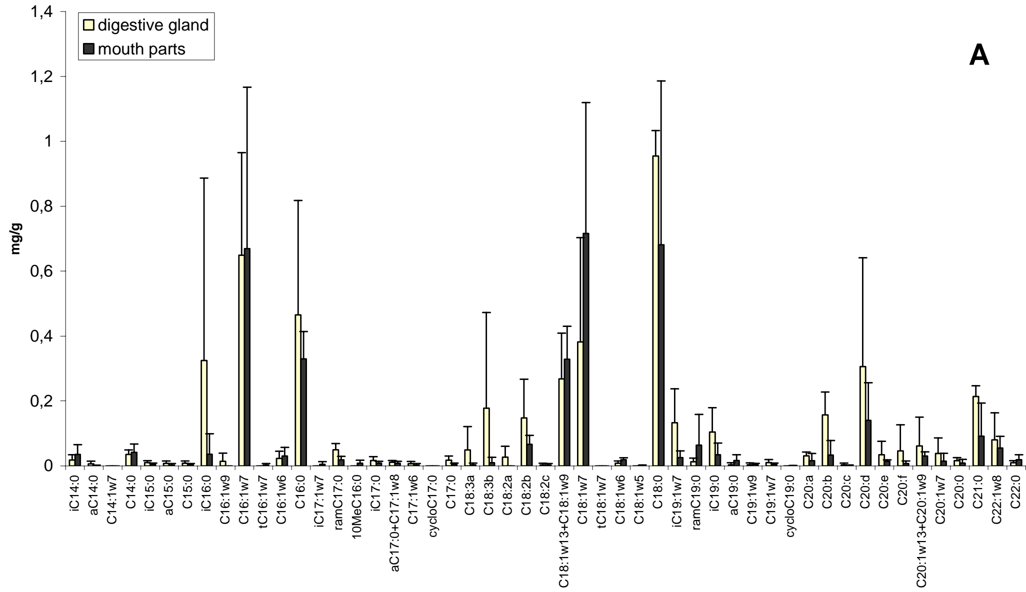

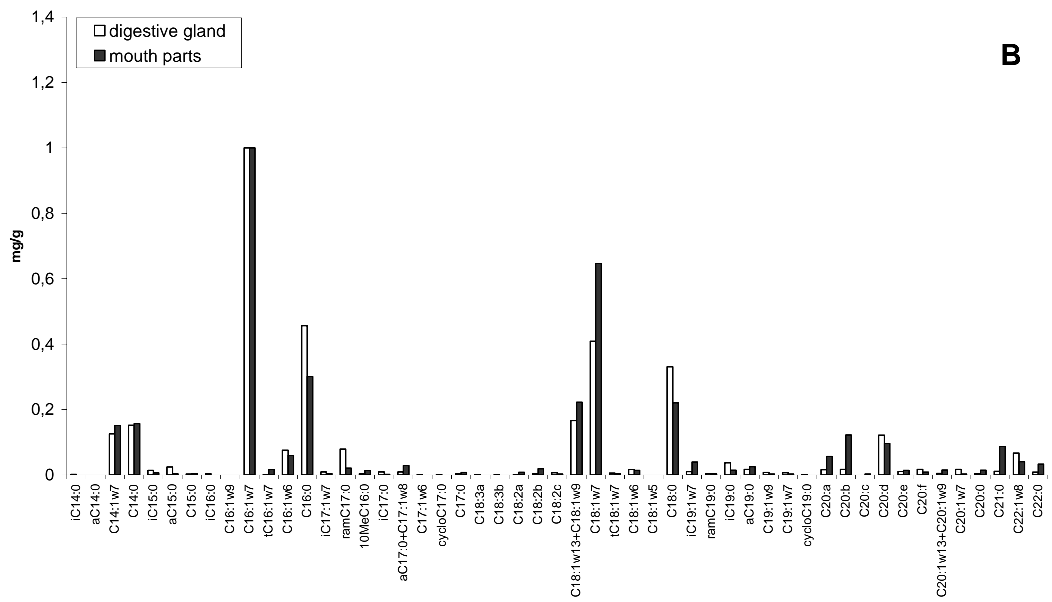

Figure 2

1 Table 1 Most abundant fatty acids biomarkers for the stated microorganisms.

Microorganism	**Biomarker**	**References**
Archaea	Glycerol Ether	Guezzenec, 1995
Phytoplankton	C20:5ω3; C20:6ω3	Chuecas & Rieley, 1969
Desulfovibrio (Sulfate reducer)	iC17:1ω7c; iC15:1ω7c; iC19:1ω7c;	Nichols *et al.*, 1986
Desulfobacter (Sulfate reducer)	10Me16; cyC18	Boon *et al.*, 1977
Thiooxidizing	C16:1ω7; C18:1ω7	McCaffrey *et al.*, 1989
Methanotrophs	C16:1ω5t; C16:1ω6; C16:1ω8; C18:1ω6; C18:1ω8	Nichols *et al.*, 1987; Bowman *et al.*, 1993
Thiobacillus sp. (Sulfur oxidizer)	iC17:1ω510-11 Me C18:1ω6;	Kerger *et al.*, 1986

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Table 2 Fatty acid composition of polar lipids expressed as a percentage of the total fatty acids for each taxonomic group. MG stands for Menez Gwen, LS for Lucky Strike; Rb for Rainbow; BS for Broken Spur; SP for Snake Pit; Lg for Logatchev and are indicative of the number of animals studied (not the number of profiles) for each vent field. “dg” stands for digestive gland. On the fatty acids, Br stands for branched.

Fatty acids	*A.lutzi* (all) **LS =2; Rb =2**	*B. seepensis* (all) **LS =8; Rb =1; SP =1**	*Bathymodiolus* spp.(dg) **MG =4; LS= 14; Rb= 4; BS=1; SP=2; Lg=1**	*R. exoculata* (dg) **LS 1; Rb= 6; BS=1; SP=2; TAG=2 Lg=1**	*M. fortunata* (dg) **LS=4; Rb=2**	*C. chacei* (dg) **LS=1; BS=1**	*Sericosura* sp. (all) **LS=2**	Amphipod (all) **MG =1; LS= 1**	*A. markensis* (dg) **SP=1**	*Phymorhynchus* sp (dg) **LS=2; Rb= 2; SP=1; TAG=1**	*S.mesatlantica* (dg) **LS= 6; Rb= 3; BS=2; TAG=2; Lg=2**
C12:0	0	0	0.01	0.09	0	0	0	0	0	0	0.04
iC14:0	0	0	2.00	0.15	0	0.06	0	0	0	6.00	0.17
aC14:0	0	0	3.00	0	0	0	0	0	0	0	7.00
C14:1W7	0	0	0	1.32	0	0	0	0	0	0	0
C14:0	0.91	0.46	0.29	2.28	1.52	0.56	0.89	0.17	0.60	0.45	1.00
iC15:0	0.24	0.09	6.00	0.32	0.14	0	0.45	0	0.28	0.08	0.21
aC15:0	0.05	0.02	0.01	0.13	0	0	0	0	0	0.05	0.04
C15:0	0.62	0.09	0.11	0.91	0.63	0.04	0	0	0.12	0.06	0.38
iC16:0	0.03	0	0.17	1.65	0.25	0	0	0	2.13	0.12	0.07
C16:1w9	1.02	0.86	0.67	8.50	0	0	0	0.43	0.30	0.14	0.72
C16:1w7	8.46	8.09	13.03	14.91	24.29	13.90	1.42	12.42	15.41	3.19	15.37
tC16:1w7	0.18	0.04	9.00	0	0	0.10	0	0.22	0	0	0.14
C16:1w6	0	0	0	0.98	0	0.11	0	0.16	0.20	0.05	0.28
C16:0	26.86	10.84	20.87	106.00	12.36	11.45	12.06	10.70	11.06	14.92	15.99
iC17:1w7	0.13	0.14	0.26	0.71	0.27	0.11	0	0.21	0	0.16	0.14
BrC17:0	1.26	0.11	0.19	1.64	0.12	0.24	0	0	0.29	1.10	0.64
10MeC16:0	0.18	0	0	0.05	0	0.21	0	0	0	0	0
iC17:0	1.00	0.14	0.13	0.51	0	0.04	0	0.17	0.17	0.04	0.26
aC17:0+C17:1w8	2.07	0.11	0.15	0.19	0.36	0.35	0	0.11	0.75	0.07	0.48
C17:1w7	0.20	0	0	0	0	0	0	0	0	0.18	0
C17:1w6	0.42	0	0	0.03	0	0	0	0	0	0	0
cycloC17:0	0	0	0.03	0	0	0	0	0	0	0	0
C17:0	0.72	0.81	0.66	0.37	0.32	0.33	0.26	0.19	0.11	0.44	0.16
C18:3(7.10.13)	0.24	8.00	0.13	0.18	0	0	0	0	0.64	2.55	0.13

C18:3(9.12.15)	0.44	0.53	4.30	0.68	0.44	0.75	0	6.97	0.97	0.03	0.13
Unknown a	0	0	0	0.21	0	0	0	0.71	0	0	0
C18:2(5.13)	0.57	0.27	0.31	2.38	0.06	0.08	0	2.58	0.17	0.37	0.14
C18:2(6.9)	2.92	0.17	0.78	0.15	2.04	0.40	0	4.19	1.41	0.73	0.47
C18:2c	2.09	1.05	0.08	0.23	0.06	0.16	0	1.01	0.37	1.24	0.45
C18:1w13+C18:1w9	6.32	6.10	2.95	5.11	5.86	11.61	3.21	13.52	27.57	2.93	18.42
C18:1w7	8.59	14.36	3.05	12.10	12.20	13.59	3.73	12.12	22.06	9.21	19.06
tC18:1w7	0	0.15	5.00	0.03	0	0	0	0	0	1.00	0.42
C18:1w6	0	0	0	0.22	0	0.15	0	0	0.71	0.27	0.50
C18:1w5	0.73	0.13	0.03	0	0	0	0	0.20	0	0.02	0
C18:0	5.96	7.90	9.04	17.51	21.75	28.30	54.54	8.36	4.20	10.05	8.64
iC19:1w7	0.07	0.08	0.13	1.48	1.57	0.64	0	0.28	0.37	0.19	0.42
Unknown b	0	0	3.00	0	0	0	0	0	0	0	0
BrC19:0	0.17	0.05	0.25	0.44	0.07	0.30	0	0.50	0	0.11	0.08
iC19:0	1.93	0.41	4.30	1.82	1.85	0.84	0	2.18	1.18	1.95	1.50
aC19:0	0	0	0	0.14	0	0	0	0	0	0	0.21
C19:1w9	0.29	1.00	0.63	0.16	0.30	0.08	0	0.16	0	0.40	0.14
C19:1w7	0.23	1.38	0.85	0.22	0	0.46	0	0.48	0.31	0.66	0.32
cycloC19:0	0	0	0	0.03	0	0	0	0	0	0	0
Unknown c	0	0	0.13	0	0	0	0	0	0	0	0
C20:3(7.10.15)	4.06	0.62	0.76	0.74	0.68	1.10	0.96	2.62	1.23	2.43	0.28
C20:3(9.12.15)	3.61	4.15	3.95	2.15	1.93	3.42	17.53	6.54	1.14	5.17	1.65
C20:2(6.15)	0.50	5.18	4.05	0.17	0.69	0.60	0	2.05	0.48	3.61	0.51
C20:2(7.15)	0.48	0.64	5.10	3.25	0.78	1.03	0	1.04	0.16	3.88	0.61
C20:2(8.15)	1.02	1.38	1.46	0.33	0.79	0.53	0	1.70	0.16	2.34	0.58
C20:2(10.15)	1.22	4.84	0.42	0.47	1.15	1.19	0.24	0.51	0.18	3.00	0.37
C20:1w13+C20:1w9	2.49	16.13	5.84	0.73	1.11	0.61	1.66	1.38	2.90	17.58	2.19
C20:1w7	7.12	7.65	4.51	0.31	1.28	1.20	2.23	2.61	1.82	5.79	1.99
C20:0	0.59	2.35	7.03	0.30	0.82	0.47	0	0.37	0.54	0.93	2.43
C21:1w9	0.77	0	0	0	0	0	0	0	0	0	0
C21:1w7	2.26	0	0	0	1.61	1.53	0	2.53	0	0	0
C21:0	1.03	1.70	3.34	2.69	1.99	0.89	0.82	0.62	0	3.32	0.98
C22:1wx	0	0	0	0.99	0.71	2.17	0	0	0	0.12	0.98
C22:0	0	0	0	0.05	0	0.41	0	0	0	0.05	0.34