CHAPTER 3: TROPHIC POSITION OF ANTARCTIC AMPHIPODS - ENHANCED ANALYSIS BY A 2-DIMENSIONAL BIOMARKER ASSAY

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ABSTRACT

The discrepancy between the ecological significance of amphipods in the Antarctic and our poor knowledge of their ecofunctional role calls for a more detailed investigation of their trophic status in this ecosystem. Twelve amphipod species from suspension-feeder to scavenger have been considered in this study. Our objective was to investigate whether the combination of fatty acid and stable isotope signatures into a 2-dimensional trophic biomarker assay would increase accuracy in the identification of Antarctic benthic amphipod trophic position. Amphipod isotopic averages ranged from –29.3‰ ($\delta^{13}C$) and 4.1‰ ($\delta^{15}N$) for the suspension-feeder *Ampelisca richardsoni*, to –21.7‰ ($\delta^{13}C$) and 11.9‰ ($\delta^{15}N$) for the high predator *Iphimediella* sp. Cluster analysis of the fatty acid composition separated the amphipod species into 4 trophic groups; suspension feeders, macro-herbivores, omnivores and scavengers. The suspension feeder was isolated due to an important proportion of 18:4(n-3), fatty acid biomarker of phytoplankton. Macro-herbivores were found to rely heavily on macroalgal carbon, containing a high percentage of arachidonic acid 20:4(n-6). Scavenger amphipods revealed a unique fatty acid composition dominated by one single fatty acid, 18:1(n-9), probably the result of a very intensive de novo biosynthesis to cope with starvation periods. Our data emphasize the need to combine different types of information to be able to draw the right conclusions regarding trophic ecology. Indeed, in some cases, the exclusive use of one type of tracing method, fatty acids or stable isotopes, would have lead to misleading/false conclusions in the trophic classification of amphipods. Therefore a 2-dimensional biomarker assay is a useful tool to elucidate the trophic positions of benthic amphipods.

KEY WORDS: 2-dimensional biomarker, trophic relationships, stable isotopes, fatty acids, Amphipoda, Antarctic ecology

3.1. INTRODUCTION

In the Southern Ocean, amphipod crustaceans are among the most specious animal group in the zoobenthos. About 530 species have been recorded as strictly Antarctic species and more than 830 species have been described so far for the whole Southern Ocean (Klages 1991, De Broyer & Jazdzewski 1996, De Broyer et al. 1999, 2003a, b, Gutt et al. 2000). It is commonly assumed that species and trophic diversity are related (Ulanowicz 2000, Dauby et al. 2001b). Also, in Antarctic waters and on Antarctic bottoms, amphipods have developed a rich variety of life styles: epontic dwellers, (bentho-) pelagic swimmers, walkers, crawlers, and burrowers. They occupy many niches reserved for decapod crustaceans in other systems (Dauby et al. 2001a, b, De Broyer et al. 2001). This diversity in life style, associated with the variety of available food, is likely to be a factor which has favoured the adaptative radiation of the Amphipoda and the diversification of trophic types in Antarctic waters (Jazdzewski et al. 1996, De Broyer et al. 2001, Dauby et al. 2001b). Regarding total energy flow in the eastern Weddell Sea shelf ecosystem, Amphipods are among the key taxa in the benthic sub-system (Jarre-Teichmann et al. 1997, Dauby et al. 2003).

Biomarkers such as fatty acids and stable isotopes have been used successfully to identify trophic relationships in marine food webs (Hobson et al. 1995, Lepoint et al. 2000, Graeve et al. 2001, Auel et al. 2002, Nyssen et al. 2002). Fatty acids are the primary constituents of most lipids. They generally remain intact through digestion and can be deposited in the consumer's tissue with minimal modification from diet and in a predictable way (Lee et al. 1971). Certain fatty acids have specific known sources and can act as biomarkers. These features make fatty acids a potential food chain tracer in marine ecosystems, which has shown its suitability in various studies (Sargent 1976, Sargent & Henderson 1986, Graeve et al. 2001, Iverson et al. 2002, Dalsgaard et al. 2003).

Stable isotope ratios also provide signatures based on actual food assimilation but are integrated over a period corresponding to the turnover time of the analysed tissues (Tieszen et al. 1983, Hobson et al. 1996, 1997). The technique relies upon the direct relationship between the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of animals and those of their diets (De Niro & Epstein 1978, 1981, Peterson & Fry 1987). Changes in ratios (i.e. fractionation) occur through metabolic processes, which cause change in the relative proportions of stable isotopes. As a result, the stable isotope composition of a consumer is indicative of and in general heavier than that of its prey. The more conservative transfer of carbon isotopic compositions (0.5‰–1‰ enrichment per trophic transfer) can be useful to trace two food sources with clear differences in their $\delta^{13}C$ values whereas nitrogen ratios (3‰-4‰ enrichment per trophic transfer) are most frequently used as trophic position indicators (Minagawa & Wada 1984, Hobson & Welch 1992, Michener & Schell 1994, Lepoint et al. 2000). However, it must be considered, that fractionation is not constant and many factors can cause variation (Gannes et al. 1997), e.g. species (e.g. De Niro & Epstein 1981), food source (Fantle et al. 1999), nitrogen dietary content (Adams & Sterner 2000) or nutritional or hydric stress (Hobson et al. 1993). Despite those problems, isotopes have been successfully applied to the Antarctic trophic web (Wada et al. 1987, Burns et al. 1998) and particularly to the pelagic fauna and the top predators of the Weddell Sea (Rau et al. 1991a, b, 1992, Schmidt et al. 2003). Only a few stable isotopic studies have been focussed on benthic communities so far (Dunton 2001, Nyssen et al. 2002). Likewise, there are limited lipid studies of Antarctic benthic amphipods (Nelson et al. 2001, Graeve et al. 2001). More work has been conducted in the Arctic (Hobson et al. 1995, Auel et al. 2002) and on Antarctic pelagic amphipods, e.g. *Themisto gaudichaudii* (Reinhardt & Van Vleet 1986, Hagen 1988, Phleger et al. 1998).

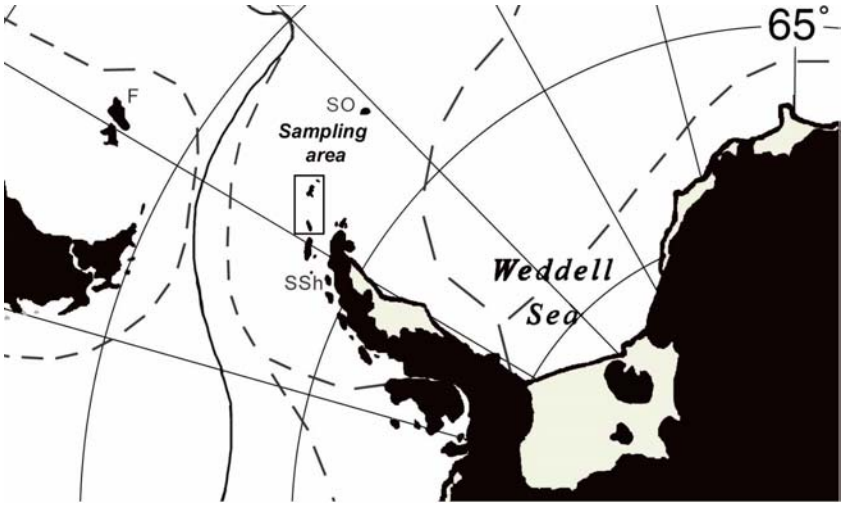
The discrepancy between the ecological significance of amphipods and our poor knowledge of their ecofunctional role calls for a more detailed investigation of their share in Antarctic trophodynamics. Furthermore, the profusion of amphipod species and the variability of their trophic spectrum in the Southern Ocean calls for a more systematic and efficient approach towards this aspect of their ecology. Our study investigates whether the combination of fatty acid and stable isotope signatures into a 2-dimensional trophic biomarker will increase accuracy in the identification of Antarctic benthic amphipod trophic position.

3.2. METHODS

3.2.1. SAMPLING AND STORAGE

The amphipods Waldeckia obesa (Chevreux 1905), Abyssorchomene plebs (Hurley 1965), Eurythenes gryllus (Lichtenstein 1822), Pseudorchomene coatsi (Chilton 1912), Epimeria similis (Chevreux 1912), Epimeria georgiana (Schellenberg 1931), Iphimediella sp, Echiniphimedia hodgsoni (Walker 1906), Eusirus perdentatus (Chevreux 1912), Djerboa furcipes (Chevreux 1906) and Ampelisca richardsoni (Karaman 1975) were caught during the cruises ANT XIX/3-4 (ANDEEP I-II), 23 January to 1 April 2002 (De Broyer et al. 2003) with RV Polarstern to the Antarctic Peninsula (Fig. 1). The animals were taken from various depths by different gear: Agassiz-trawls, bottom-trawls and autonomous traps. Immediately after sampling, individuals were sorted into species and kept for several hours in aquaria. Thereafter, individuals dedicated to isotope analyses were rinsed in distilled water and transferred into glass vials. Specimens for lipid analysis were transferred into glass vials and covered with dichloromethan:methanol (2:1, by vol.) All samples were stored at -30°C until analysis at the Alfred Wegener Institute at Bremerhaven.

Fig.3.1. Detailed map of the Antarctic Peninsula and the sampling area: F—Falklands, SO—South Orkneys, SSh—South Shetlands.



3.2.2. STOMACH CONTENT ANALYSIS

Gut contents of 20 specimens from each species preserved in 4% formaldehyde solution were examined. The digestive tract was removed from the animal, opened and the content was spread on a micro slide. The slide was examined microscopically (Leica DMLB with reflection contrast system) and every food item was determined as precisely as possible. Additional data were taken from Nyssen et al. (2002) and Dauby et al. (2001b) where the methodological details are described. Observations of feeding behaviour of the various amphipod species in aquaria provided further information on diet and feeding.

3.2.3. LIPID ANALYSIS

Lipid analyses carried out on all sampled amphipod species (n=11). Fatty acid data from Graeve et al. (2001) referring to the species *A. richardsoni*, *E.*

hodgsoni, *Oradarea edentata*, *E. georgiana* (one specimen) and *E. perdentatus* were added to our data set for comparison.

Samples stored in chloroform:methanol (2:1 by vol.) were evaporated with nitrogen to dryness and subsequently lyophilised for 48 h. Dry mass (DM) was determined gravimetrically. Total lipid mass (TL) was measured gravimetrically after lipid extraction from the freeze-dried samples using dichloromethane:methanol (2:1 by vol.), essentially after Folch et al. (1957).

Fatty acid composition was analysed by gas-liquid chromatography (Kattner & Fricke 1986). Fatty acids of the total lipid extracts were converted to their methyl esters by transesterification in methanol containing 3% concentrated sulphuric acid at 80°C for 4 hours. After extraction with hexane, fatty acid methyl esters were analysed with a Hewlett-Packard 6890 Series gas chromatograph with a DB-FFAP fused silica capillary column (30 m x 0.25 mm inner diameter; 0.25 µm film thickness) using temperature programming (160-240°C at 4°C min^{-1}, hold 15 min). For recording and integration Class-VP software (4.3) (Shimadzu, Germany) was used. Fatty acids were identified with commercial and natural standard mixtures and if necessary, additional confirmation was carried out by gas chromatography-mass spectrometry.

3.2.4. STABLE ISOTOPE ANALYSIS

Carbon and nitrogen isotopic ratios were measured in all sampled amphipod species (n=11, no isotopic data available for *O.edentata*) as well as in the brown algae *Desmarestia mensiezii*. Isotopic data for suspended particulate organic matter (SPOM) are from Nyssen et al. (2002). Muscle tissues or whole animals of small species were dried and ground with mortar and pestle into a homogenous powder. Isotopic ratios were measured individually in each specimen. Stable carbon and nitrogen isotope ratios were analysed with a nitrogen-carbon elemental analyser (Fisons, UK) directly coupled to an Optima (Micromass, UK) continuous flow isotope ratio mass spectrometer

(CF-IRMS) for combustion and automated analysis. Isotopic ratios are expressed in δ values as the proportional deviation of the sample isotope ratio from that of an international Vienna Peedee Belemnite (V-PDB) standard according to the following formula:

δX (‰) = [Rsample-Rstandard /Rstandard] x 1000,

where X is ^{13}C or ^{15}N, R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the appropriate standards were Vienna Peedee Belemnite (V-PDB) and atmospheric nitrogen for carbon and nitrogen, respectively. Intercomparison materials were IAEA-N1 ($\delta^{15}N$= +0.4 ± 0.2‰) and IAEA CH-6 (sucrose) ($\delta^{13}C$= -10.4 ± 0.2‰). Experimental precision (based on the standard deviation of replicates of an atropina standard) was 0.3‰ for both carbon and nitrogen.

3.2.5. DATA ANALYSIS

Multivariate analyses of the fatty acid composition were performed for all individuals using the program PRIMER (Plymouth Routines in Multivariate Ecological Research), Version 5 (Clarke & Warwick 1994). Hierarchical clustering and multi-dimensional scaling (MDS) were performed based on a Bray-Curtis similarity coefficient applied to untransformed percentage composition data. No transformation was applied to the data set, because those fatty acids that contribute only to a small percentage of the total composition did not feature heavily in the diet. Giving artificial weight to these minor fatty acids by applying a transformation would therefore be inappropriate. Data from Graeve et al. (2001) referring to the species *A. richardsoni*, *E. hodgsoni*, *Oradarea edentata*, *E. georgiana* (one specimen) and *E. perdentatus* were added to our data set for comparative analysis.

The SIMPER (SIMilarity PERcentage–species contribution) routine in PRIMER was used to investigate the clusters found by both hierarchical cluster analysis and MDS.

Parametric tests were used to compare isotope ratios between different taxa. Normality of the data was checked by the Kolmogorov-Smirnov test followed by ANOVA and post-hoc (Tukey test) comparisons of means. A significance level of $p < 0.001$ was used in all tests (Scherrer 1984) except when it is mentioned.

3.3. RESULTS

3.3.1. STOMACH CONTENT & TROPHIC TYPE

Major stomach contents and corresponding trophic type of the 11 amphipod species are summarized in Table 3.1. Detailed stomach content data are provided by Dauby et al. (2001b) and Nyssen et al. (2002). Trophic type of the 11 species ranged from suspension feeder to scavenger.

Table 3.1. Classification of 11 species of Antarctic amphipods in different trophic categories following the composition of their stomach contents (Dauby et al. 2001b, Nyssen et al. 2002, this study)

Species	Trophic type	Major prey
Ampelisca richardsoni	Suspension feeder	Phytoplancton
Djerboa furcipes	Herbivore	Brown Macroalgae
Epimeria similis	Micropredator	Hydrozoan
Epimeria georgiana	Deposit feeder	Detritus
Eusirus perdentatus	Predator	Crustaceans
Echiniphimedia hodgsoni	Micro predator	Sponges
Iphimediella sp	Predator	Crustaceans
Pseudorchomene coatsi	Scavenger	Carrion
Abyssorchomene plebs	Scavenger	Carrion
Eurythenes gryllus	Scavenger	Carrion
Waldeckia obesa	Scavenger	Carrion

3.3.2. FATTY ACID COMPOSITION

The fatty acid composition, albeit different between species, showed some overall similarities (Table 3.2). The principal fatty acids of all species were 16:0, 18:1 (both isomers), 20:4(n-6), 20:5(n-3) and 22:6(n-3). High percentages of polyunsaturated fatty acids (PUFA) were found in *A. richardsoni* (58%) whereas monounsaturated fatty acids (MUFA) were most abundant in *E. gryllus*, accounting for up to 58%. The hierarchical cluster analysis separated twelve amphipod species into 5 distinct groups at the 80% similarity level (Fig. 3.2, see p.21). Clusters C1 and C5 are mono-specific and Cluster 4 is well separated into single species groupings. In Clusters C2 and C3 the individuals are not gathered by species in subgroups but more spread, although some separation was still apparent. *Iphimediella* sp. and one specimen of *E. hodgsoni* remained outside the clusters defined at the 80% similarity level: As shown by the SIMPER analysis (Table 3.3), these groupings had high, within group, similarities. The statistical treatment, using all fatty acids for each group indicated that essentially the oleic acid (18:1(n-9)) distinguished Cluster 1 (*W. obesa*) from all other clusters. The fatty acid profile of *W. obesa* was unique since oleic acid accounted for more than 44% of total fatty acids. This unusually high proportion of oleic acid is responsible for the split of scavenger species into two different clusters (C1 and C2). The SIMPER analysis revealed also that it is mainly the higher proportion of the fatty acid 18-4(n-3) which isolates Cluster 5 from the other Clusters. The highest levels of C_{18} and C_{20} PUFA (mainly arachidonic acid (20:4(n-6)), which is the discriminant fatty acid for this cluster) occurred in Cluster 4 (*D. furcipes* and *O. edentata*). Besides all the clusters, the isolated position of the iphimediid species in the dendrogram seems to be due to its considerably high levels of 20:1 and 22:1 fatty acids (19% in total).

Table 3.2. Fatty acid composition (mean value ± SD) of total lipid extracted from 12 species of amphipods from the Southern Ocean. Only values ≥ 0.3% are mentioned. Number of analysed individuals in brackets. Wo—*Waldeckia obesa*, Ap—*Abyssorchomene plebs*, Eg—*Eurythenes gryllus*, Pc—*Pseudorchomene coatsi*, Es—*Epimeria similis*, Ege—*Epimeria georgiana*, Eh—*Echiniphimedia hodgsoni*, Ep—*Eusirus perdentatus*, Df—*Djerboa furcipes*, Oe—*Oradarea edentata* (data from Graeve et al. (2001), Ar—*Ampelisca richardsoni*, Iphi—*Iphimediella* sp

Fatty acids	*Wo* (7)	*Ap* (9)	*Eg* (2)	*Pc* (1)	*Es* (2)	*Ege* (2)	*Eh* (2)	*Ep* (1)	*Df* (2)	*Oe* (2)	*Ar* (3)	Iphi *(1)*
14:0	11.3±2.9	3.4 ±1.0	1.7 ±0.9	6.9	1.3 ±0.1	1.1 ±0.1	0.7 ±0.1	2.1	1.0 ±0.1	1.6 ±0.1	5.3 ±0.2	3.5
15:0	0.8 ±0.9	0.3	0.2 ±0.1	0.4	1.2 ±0.4	0.9 ±0.4	0.7 ±0.1	0.4	0.6 ±0.1	0.6 ±0.2	0.1	0.4
16:0	12.9 ±1.6	12.9 ±2.0	11.0 ±2.9	22.5	13.4 ±3.5	11.4 ±1.9	8.9 ±1.1	15.3	16.8 ±1.1	14.7	8.8 ±0.6	12.5
17:0	0.4 ±0.3	0.9 ±0.8	0.1 ±0.1	0.2	0.5 ±0.1	0.3 ±0.3	0.1	0.1	2.0 ±2.8	0.1	0.1	0.4
18:0	1.9 ±0.8	1.1 ±0.3	1.8 ±0.2	2.9	1.1 ±0.1	1.1 ±0.5	1.1 ±0.1	0.9	1.7 ±0.1	0.5 ±0.1	1.2 ±0.2	3.0
16:1(n-7)	6.7 ±1.4	10.0 ±3.2	7.5 ±0.6	7.9	2.1 ±0.4	3.6 ±1.3	11.4 ±1.5	3.7	3.1 ±0.3	7.4 ±1.4	9.1 ±1.9	3.9
18:1(n-9)	44.1 ±2.7	30.5 ±4.9	33.9 ±2.7	31.1	20.2 ±1.6	21.9 ±0.2	19.9 ±5.2	22.7	17.7 ±1.1	20.9 ±0.7	8.3 ±0.7	19.7
18:1(n-7)	2.9 ±2.1	6.8 ±0.9	7.4 ±0.6	6.2	6.1 ±0.2	8.4 ±0.5	10.6 ±3.6	5.2	3.3 ±0.2	5.1 ±1.1	3.3	6.1
20:1(n-9)	1.1 ±0.5	5.1 ±2.9	4.9 ±0.3	2.6	1.9	1.8 ±0.7	1.1	1.3	1.0 ±1.3	1.6 ±0.1	1.4 ±0.2	9.3
20:1(-7)	0.3 ±0.1	0.9 ±0.3	1.9 ±0.7	0.5	1.0 ±0.7	2.3 ±0.4	2.8 ±0.9	0.7	0.0	0.3 ±0.3	0.6 ±0.1	5.8
22:1(n-11)	0.6 ±0.6	1.5 ±1.0	1.7 ±1.5	2.5	0.3 ±0.2	0.4 ±0.2	0.2 ±0.1	0.2	0.6 ±0.8	0.3 ±0.2	0.1	3.5
22:1(n-9)	0.1 ±0.1	0.6 ±0.7	0.5 ±0.6	0.1	0.5 ±0.4	0.4 ±0.1	0.7 ±0.4	0.4	0.0	0.3	0.1	0.1
16:2(n-4)	0.5 ±0.2	0.6 ±0.9	3.2 ±4.2	1.2	0.7 ±0.1	0.6 ±0.1	0.6 ±0.7	0.5	1.8 ±0.1	2.3 ±0.3	2.1 ±0.5	1.6
18:2(n-6)	1.0 ±0.3	1.8 ±0.3	1.8 ±0.4	1.1	1.7 ±0.1	1.8 ±0.3	4.7 ±2.2	2.2	5.4 ±0.4	3.6 ±0.2	2.0 ±0.2	1.2
16:3(n-4)	1.0 ±0.2	0.4 ±0.1	0.5 ±0.1	0.5	0.6 ±0.5	0.8 ±0.2	1.1 ±0.1	0.5	0.3 ±0.4	0.8 ±0.1	0.4 ±0.1	0.5

Fatty acids	*Wo* (7)	*Ap* (9)	*Eg* (2)	*Pc* (1)	*Es* (2)	*Ege* (2)	*Eh* (2)	*Ep* (1)	*Df* (2)	*Oe* (2)	*Ar* (3)	Iphi *(1)*
16:4(n-1)	0.1	0.2 ±0.1	0.8 ±0.8	0.1	0.0	0.1	0.1	0.1	0.0	0.1	1.4 ±0.6	0.1
18:3(n-6)	0.2	0.2	0.5 ±0.1	0.1	0.2 ±0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.2
18:3(n-3)	0.2	0.5 ±0.1	0.5 ±0.2	0.4	0.5 ±0.2	0.5 ±0.3	0.8 ±0.2	0.8	3.3 ±0.5	2.7 ±0.3	1.2	0.3
18:4(n-3)	0.3 ±0.1	0.8 ±0.4	0.6 ±0.2	0.8	0.7 ±0.6	0.7 ±0.5	0.3 ±0.3	1.7	1.8 ±0.4	1.9 ±0.5	21.4	0.3
20:4(n-6)	1.3 ±0.5	1.4 ±1.7	1.4 ±0.4	1.1	8.0 ±3.3	8.2 ±2.7	2.7 ±1.2	2.8	15.0 ±0.9	20.0 ±1.5	0.7	1.4
20:4(n-3)	0.1	0.5 ±0.2	0.6 ±0.2	0.5	0.4 ±0.4	0.3 ±0.1	0.1	0.4	0.7 ±0.1	0.8 ±0.1	3.1 ±0.4	0.5
20:5(n-3)	6.1 ±1.5	9.1 ±2.4	8.7 ±2.5	4.3	19.0 ±1.6	19.2 ±2.0	16.7 ±3.2	19.7	19.9 ±1.3	12.4 ±0.5	19.2 ±0.6	14.6
22:5(n-3)	0.6 ±0.8	1.6 ±0.8	0.4 ±0.1	1.0	0.8 ±0.3	1.2 ±0.1	0.3 ±0.3	0.7	0.8	1.3 ±0.2	0.1	2.9
22:6(n-3)	6.0 ±1.0	8.9 ±2.1	8.8 ±1.7	5.4	18.1 ±2.6	13.2 ±0.3	14.9 ±11.3	17.7	2.7 ±0.3	0.9 ±0.1	10.5 ±0.8	8.3
sum PUFA	15.8 ±4.4	23.6 ±7.9	22.7 ±6.4	14.2	48.2 ±9.6	44.2 ±6.3	37.1 ±16.6	44.5	44.3 ±3.8	41.1 ±3.3	58.0 ±2.5	29.1
C18 ratio	15.0	4.5	4.6	5.0	3.3	2.6	1.9	4.3	5.4	4.1	2.5	3.3
sum 20:1	1.4 ±0.7	6.0 ±3.1	6.8 ±1.0	3.1	2.9 ±0.7	4.1 ±1.1	3.9 ±0.9	1.9	1.0 ±1.3	1.9 ±0.4	2.0 ±0.3	15.0
sum 22:1	0.7 ±0.7)	2.1	2.1 ±2.1	2.6	0.8 ±0.6	0.8 ±0.3	0.9 ±0.6	0.6	0.6 ±0.8	0.6 ±0.3	0.2	3.6

3.3.3. STABLE ISOTOPE RATIOS

The average carbon and nitrogen isotope ratios range from –29.3‰ ($\delta^{13}C$) and 4.1‰ ($\delta^{15}N$) in *A. richardsoni* to –21.7‰ ($\delta^{13}C$) and 11.9‰ ($\delta^{15}N$) in *Iphimediella* sp (Table 3.4). The inter-species differences are significant as indicated by ANOVA and subsequent post-hoc tests (Tables 3.5a & 3.5b). Displaying the lowest isotopic ratios, *A. richardsoni* ($\delta^{13}C$ = -27.1 ± 0.9‰; $\delta^{15}N$ = 6.6 ± 0.6‰) and *D. furcipes* ($\delta^{13}C$ = -23.4 ± 0.6‰; $\delta^{15}N$ = 4.9 ± 0.3‰) resemble primary producers, i.e. the suspended particulate organic matter and the brown macroalgae *Desmarestia mensiezii*. The isotopic ratios of these primary consumers are significantly different from values of all the other species (Tukey test, $p<0.001$).

Both Epimeriidae and the species *E. perdentatus* show wide ranges of isotopic ratios. As illustrated in Figure 3.3 (see p.24), the range of values is wider for $\delta^{13}C$ than for the $\delta^{15}N$. The difference between maximum and minimum $\delta^{13}C$ is from 2.5 to 5.5‰. This difference is less pronounced for nitrogen (from 1.5 to 3‰). The species displaying the widest range of values is *E. georgiana.* The scavengers are clearly separated into two groups and this scission is essentially due to their significantly different $\delta^{13}C$ (Tukey test, $p<0.001$). The first group is composed of the lipid-rich species *A. plebs* and *E. gryllus* while the second gathers the lipid-less *W. obesa* and *P. coatsi* (Nyssen & Graeve, unpublished results).

Table 3.4. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of 11 species of Antarctic amphipods (mean ± SD); n: number of samples.

Species	N	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
Ampelisca richardsoni	3	-29.3 ± 0.2	4.1 ± 0.1
Djerboa furcipes	5	-27.8 ± 0.6	4.9 ± 0.3
Eusirus perdentatus	14	-23.4 ± 0.6	7.3 ± 1.0
Epimeria similis	15	-25.0 ± 1.5	7.6 ± 0.5
Epimeria georgiani	17	-23.7 ± 1.7	7.9 ± 0.4
Echiniphimedia hodgsoni	2	-24.3 ± 1.3	10.6 ± 1.8
Iphimediella sp	4	-21.7 ± 1.2	11.9 ± 0.9
Pseudorchomene coatsi	3	-22.7 ± 0.3	9.3 ± 0.3
Abyssorchomene plebs	6	-26.6 ± 0.5	9.5 ± 0.8
Eurythenes gryllus	9	-27.3 ± 1.1	8.5 ± 0.5
Waldeckia obesa	5	-22.8 ± 0.9	7.3 ± 0.7

The highest positioned species in the food web, *Iphimediella* sp. displays significantly different $\delta^{15}N$ to the other species (Tukey test, $p<0.001$) except from *E. hodgsoni* which belongs to the same family. However, the $\delta^{13}C$ value shows some similarity with other species, such as *W. obesa*, *E. perdentatus*, *P. coatsi and E. georgiana*.

Table 3.3. Results of SIMPER analysis: within-group similarity (% in parenthesis), average dissimilarity (%) and separating fatty acids (FA) (most discriminant).

Average Dissimilarity + separating FA	CLUSTER 1 (89.1%)	CLUSTER 2 (83.7%)	CLUSTER 3 (81.8%)	CLUSTER 4 (85.3%)	CLUSTER 5 (95.0%)
CLUSTER 1	-	25.4% 18:1(n-9)/14:0	41.1% 18:1(n-9)/20:5(n-3)	44 .3% 18:1(n-9)/20:4(n-6)	50.5% 18:1(n-9)/18:4(n-3)
CLUSTER 2		-	29.7% 18:1(n-9)/20:5(n-3)	36.8% 20:4(n-6)/18:1(n-9)	43.2% 18:1(n-9)/18:4(n-3)
CLUSTER 3			-	29.9% 22:6(n-3)/20:4(n-6)	36.7% 18:4(n-3)/18:1(n-9)
CLUSTER 4				-	44.5% 18:4(n-3)/20:4(n-6)
CLUSTER 5					-

Table 3.5a. ANOVA results: post-hoc test (Tukey test) for $\delta^{13}C$. "x" indicates significant with $p < 0.001$, "x*" indicates significant with $p < 0.005$ and "ns" indicates no significant difference between means at $\alpha = 0.05$.

	Species	N	$\delta^{13}C$	1	2	3	4	5	6	7	8	9	10	11
1	*A. richardsoni*	3	-29.3±0.2		ns	x	x	x	x	x	x*	ns	x	x
2	*D. furcipes*	5	-27.8 ± 0.6			x	x	x	x*	x	ns	ns	x	x
3	*E. similis*	15	-25.0 ± 1.5				x	x*	ns	x	ns	x	ns	x*
4	*E. georgiana*	17	-23.7 ± 1.7					ns	ns	ns	x	x	ns	ns
5	*E. perdentatus*	14	-23.4 ± 0.6						ns	ns	x	x	ns	x*
6	*E. hodgsoni*	2	-24.3 ± 1.3							ns	ns	x*	ns	ns
7	*Iphimediella* sp.	4	-21.7 ± 1.2								x	x	ns	ns
8	*A. plebs*	6	-26.6 ± 0.5									ns	x	x
9	*E. gryllus*	9	-27.3 ± 1.1										x	x
10	*P. coatsi*	3	-22.7 ± 0.3											ns
11	*W. obesa*	5	-22.8 ± 0.9											

Table 3.5b. ANOVA results: post-hoc test ±Tukey test) for $\delta^{15}N$. "x" indicates significant with $p < 0.001$, "x*" indicates significant with $p < 0.005$ and "ns" indicates no significant difference between means at $\alpha = 0.05$, n: number of samples.

	Species	N	$\delta^{15}N$	1	2	3	4	5	6	7	8	9	10	11
1	*A. richardsoni*	3	4.1 ± 0.1		ns	x	x	x	x	x	x	x	x	x
2	*D. furcipes*	5	4.9 ± 0.3			x	x	x	x	x	x	x	x	x
3	*E. similis*	15	7.6 ± 0.5				ns	ns	x	x	x	ns	ns	ns
4	*E. georgiana*	17	7.9 ± 0.4					x	ns	x	ns	ns	ns	ns
5	*E. perdentatus*	14	7.3 ± 1.0						x	x	x	ns	x	ns
6	*E. hodgsoni*	2	10.6 ± 1.8							ns	ns	ns	ns	x
7	*Iphimediella* sp.	4	11.9 ± 0.9								x	x	x*	x
8	*A. plebs*	6	9.5 ± 0.8									ns	ns	x*
9	*E. gryllus*	9	8.5 ± 0.5										ns	ns
10	*P. coatsi*	3	9.3 ± 0.3											ns
11	*W. obesa*	5	7.3 ± 0.7											

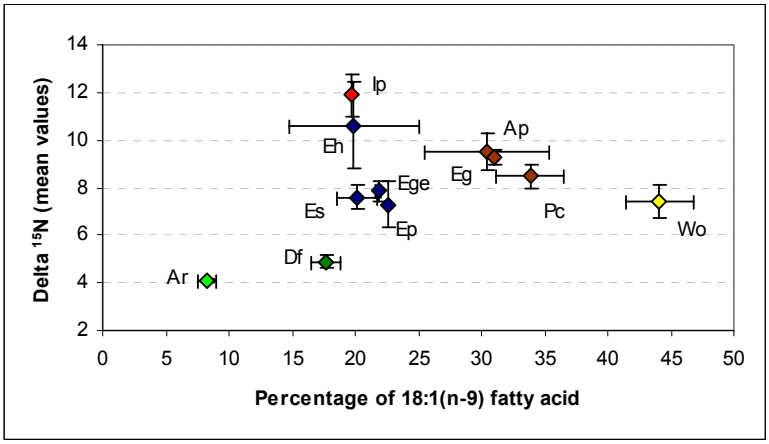
3.3.4. THE 2-DIMENSIONAL BIOMARKER APPROACH

In order to check whether the combination of fatty acid and stable isotope data is useful to enhance the identification of trophic positions, $\delta^{15}N$ values were plotted versus four fatty acid types which are characteristic biomarkers for certain food types or feeding strategies (Figs 3.4a to 3.4b).

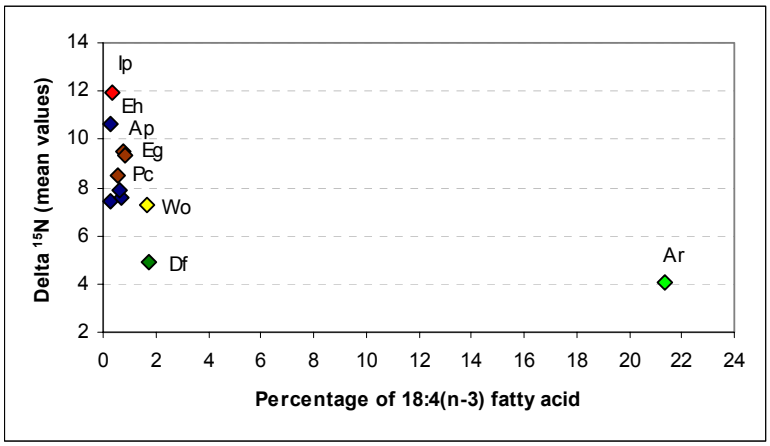
18:1(n-9) fatty acid is considered to be a signature of carnivory (Graeve et al. 2001, Auel et al. 2002). There is a general positive relationship between $\delta^{15}N$ and 18:1(n-9) (Fig 3.4a). The negative relationship between $\delta^{15}N$ and the polyunsaturated fatty acid 18:4(n-3), recognized as a biomarker of haptophytes (Graeve et al. 1994a, b), is illustrated in Figure 3.4b. The distinction between primary consumers food preferences is evident from comparison of Figures 3.4b and 3.4c. Finally, the plot of 20:1 and 22:1 fatty acids, synthesized only by calanoid copepods (Graeve et al. 1994a, b, Hagen et al. 1993, 2000, Kattner et al. 1994), against $\delta^{15}N$ shows a clear positive correlation (Fig. 3.4d).

Figs.3.4a to 3.4d. Nitrogen isotopic ratios plotted *vs* concentration of fatty acid biomarkers (% of total fatty acids) of 11 species of Antarctic amphipods: Wo—*Waldeckia obesa*, Ap—*Abyssorchomene plebs*, Eg—*Eurythenes gryllus*, Pc—*Pseudorchomene coatsi*, Es—*Epimeria similis*, Ege—*Epimeria georgiana*, Ep—*Eusirus perdentatus*, Ip—*Iphimediella* sp., Eh—*Echiniphimedia hodgsoni*, Ar—*Ampelisca richardsoni*, Df—*Djerboa furcipes*.

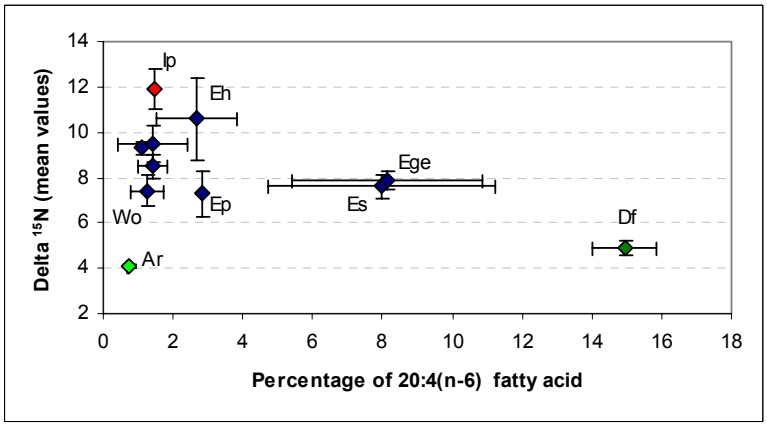
3.4a



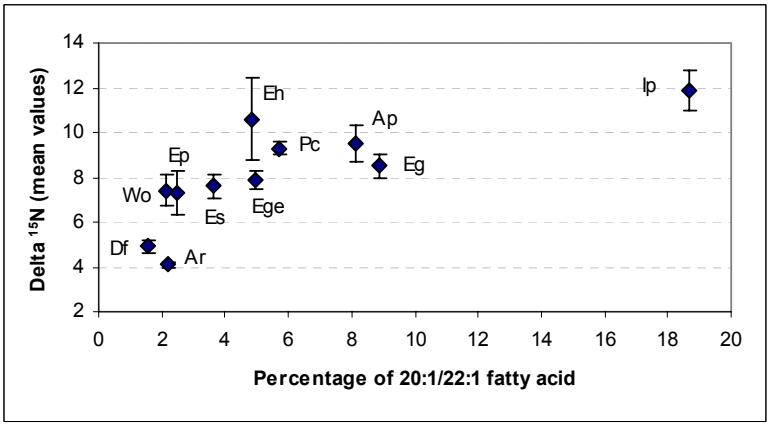
3.4b


Delta ¹⁵N (mean values)
Percentage of 18:4(n-3) fatty acid
Ip
Eh
Ap
Eg
Pc
Wo
Df
Ar

3.4c


Delta ¹⁵N (mean values)
Percentage of 20:4(n-6) fatty acid
Ip
Eh
Ege
Es
Ep
Wo
Df
Ar

3.4d

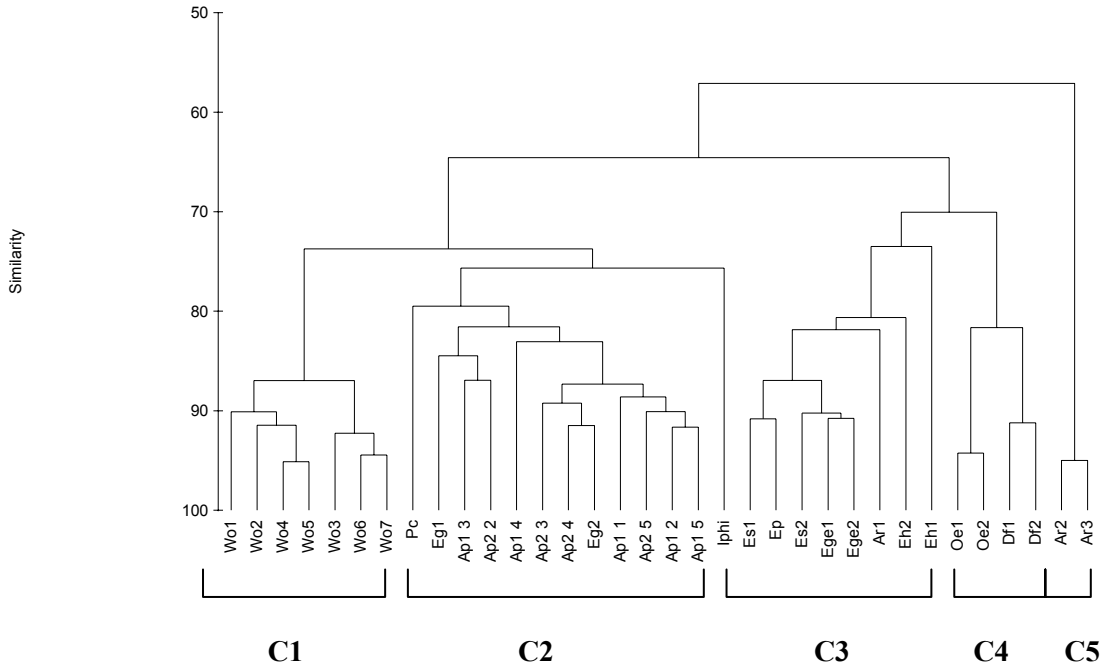

Delta ¹⁵N (mean values)
Percentage of 20:1/22:1 fatty acid
Ip
Eh
Ap
Pc
Eg
Ep
Wo
Es
Ege
Df
Ar

3.4. DISCUSSION

SIMPER analysis involving all fatty acids revealed essentially the oleic acid, to distinguish Cluster 1 from all other clusters. The fatty acid signature of *W. obesa* is characterized by extremely high levels of 18:1(n-9) and high levels of 14:0 compared to all other species. This unusual amount of 18:1(n-9) has already been recorded by Graeve et al. (2001) for the same species. Oleic acid is a major end product of the fatty acid biosynthesis in vertebrates and invertebrates. For example, Iverson et al. (2002) have reported concentrations of more than 30% of this fatty acid in Alaskan eulachon (*Thaleichthys pacificus*). In Antarctic waters, the notothenioid fishes, such as the icedevil, *Aethotaxis mitopteryx*, and the silverfish, *Pleurogramma antarcticum*, also display rather high levels of 18:1(n-9) fatty acid (about 25% of the total fatty acid composition) (Hagen et al. 2000) but none of them have ever been found to contain concentrations as high as those recorded in scavenging amphipods. The fatty acid 18:1(n-9), typically occurring in metazoans, is generally considered as a signature of carnivorous feeding (Sargent & Henderson 1986, Falk-Petersen et al. 1990, Graeve et al. 1994b, 1997, Hagen & Kattner 1998, Auel et al. 2002). Plotted against $\delta^{15}N$, which is a trophic indicator, a general positive correlation is observed, and an accumulation of 18:1(n-9) from the diet could be suggested. However, a particularly high *de novo* biosynthesis of 18:1(n-9) could also explain those high concentrations in Lysianassidae in general and *W. obesa* in particular. These fatty acids could have been synthesized by amphipods in response to short periods of satiety followed by long periods of starvation, a common situation for scavengers. Cluster 2, comprising the other scavengers, *A. plebs*, *E. gryllus* and *P. coatsi*, is also characterized by high levels of 18:1(n-9) but to a lesser extent compared to *W. obesa*. This difference, associated with the different levels of 14:0 fatty acid, is responsible for 40% of the separation of scavenger amphipods in two different clusters.

Considering the isotopic results, the species *A. plebs* and *E. gryllus* are characterized by particularly low $\delta^{13}C$ values compared to the other scavengers *W. obesa*, and *P. coatsi*. This depletion in carbon is probably due to the higher lipid content of *A. plebs* and *E. gryllus* (Nyssen & Graeve, unpublished results). Lipids are isotopically lighter than proteins and so high lipid content generally results in a decrease of the $\delta^{13}C$ of the whole body (DeNiro & Epstein 1977, Tieszen et al. 1983, Wada et al. 1987, Pinnegar & Polunin 1999, Nyssen et al. 2002).

Fig 3.2. Hierarchical cluster analysis of fatty acid composition (%) of the total lipid extracted from 12 species of Antarctic amphipods: Wo—*Waldeckia obesa*, Ap—*Abyssorchomene plebs*, Eg—*Eurythenes gryllus*, Pc—*Pseudorchomene coatsi*, Es—*Epimeria similis*, Ege—*Epimeria georgiana*, Eh—*Echiniphimedia hodgsoni*, Ep—*Eusirus perdentatus*, Df—*Djerboa furcipes*, Oe—*Oradarea edentata* (data from Graeve et al. (2001)), Ar—*Ampelisca richardsoni*, Iphi—*Iphimediella* sp.



All these scavenging amphipods belong to the family of the Lysianassidae and the conservation of a similar fatty acid composition in all of these congeners is particularly striking. A potential link between phylogeny and fatty acid composition in Lysianassids would be an interesting topic in itself. Indeed, the fatty acid composition of another Antarctic scavenger, the isopod Natatolana sp., is distinctly different despite its almost identical feeding strategy and prey spectrum (Nyssen, unpublished data).

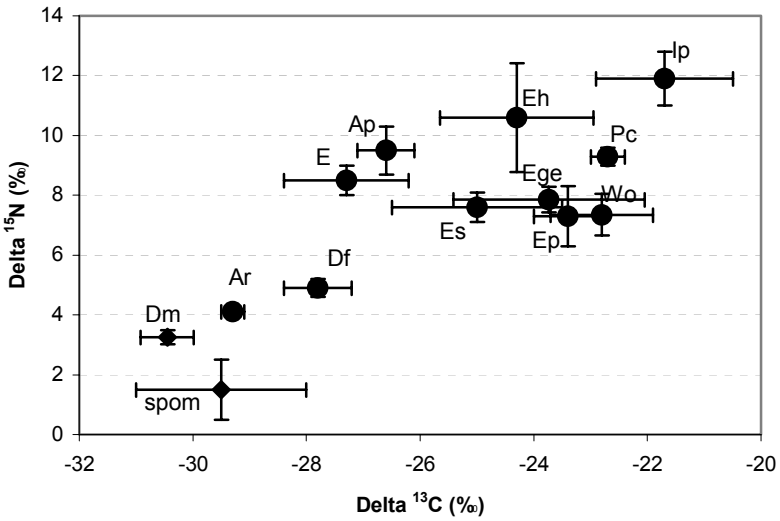
The high levels of C_{18} and C_{20} PUFAs (mainly arachidonic acid 20:4(n-6)) recorded in *D. furcipes* and *O. edentata* (Cluster 4, Fig 3.2) are well in accordance with their herbivorous diet. High concentrations of C_{18} and C_{20} polyunsaturated fatty acids have been shown to be typical of many macroalgae (Kayama et al. 1989, Cook et al. 2000, Graeve et al. 2001, Kharlamenko et al. 2001). Furthermore, judging by stomach content results, the brown alga *Desmarestia menziesii* seems to be preferentially consumed by these herbivorous amphipods. The results are corroborated by the fatty acid composition of the macroalgae, which are dominated by 20:4(n-6), 18:1(n-9) and C_{18} PUFAs (Nyssen, unpublished results). When plotted against the $\delta^{15}N$ of all species, the percentage of 20:4(n-6) displays a negative correlation; its concentration increases with decreasing ranking of the various species in the food web (Fig. 3.4c). Although they are not macroherbivore, both Epimeriidae species accumulate significant quantities of 20:4(n-6) with up to 8%. Although Graeve et al. (2002) suggested arachidonic acid as indicating a macroalgal origin; other authors have suspected protists in the sediment to be one of the sources of 20:4(n-6) (Bell & Sargent 1985, Fullarton et al. 1995, Howell et al. 2003). The presence of sediment in the stomach of *E. similis* and *E. georgiana* has already suggested at least a partial deposit feeding behaviour and 20:4(n-6) levels could reflect some assimilation of the sediment-associated micro-organisms. Furthermore, even with a significant amount of arachidonic acid, the intermediate nitrogen ratios of both Epimeriidae provide additional evidence of the distance to this fatty acid signature source. These species do probably not belong to a well-defined

trophic category but are able to modulate their feeding behaviour in response to food availability. The combination of the different approaches used here enables the classification of those epimeriid species into the wrong trophic category to be avoided. This omnivory is corroborated by the wide range of their $\delta^{13}C$ which could reflect the large spectrum of organic matter sources upon which they can rely.

The SIMPER analysis also revealed that it is mainly the higher concentration of 18:4(n-3) fatty acid which isolates *A. richardsoni* from the other amphipods. These levels attest to a major dietary input of material originating from phytoplankton such as cryptophytes and/or haptophytes (Harrington et al. 1970, Nichols et al. 1991, Graeve 1993, Graeve et al. 1994a, b, Swadling et al. 2000, Graeve et al. 2001). Figure 3.4b clearly illustrates the drastic decrease of $\delta^{15}N$, indicator of the trophic position, along with the increase of the proportions of 18:4(n-3), a biomarker for the assimilation of fatty acid of phytoplankton origin (Harrington et al. 1970, Nichols et al. 1991, Graeve 1993, Graeve et al. 1994a, b, Swadling et al. 2000, Graeve et al. 2001). In this case, confusion would have been caused by the use of stable isotopes alone to determine trophic links. If the $\delta^{15}N$ values indicate *A. richardsoni* and *D. furcipes* as primary consumers, their respective fatty acid profiles reveal that they do not rely on the same primary producers at all.

The rather isolated position of *Iphimediella* sp. (Fig.3.2) seems to be due to the significant proportions of both isomers of the long-chain monounsaturated 20:1 and 22:1 fatty acids. These long-chain monounsaturates are typical components of dominant Antarctic copepod species *Calanoides acutus* and *Calanus propinquus* (Hagen et al. 1993, Kattner et al. 1994, Hagen et al. 2000). The significance of these copepod biomarkers in the fatty acid pattern would put *Iphimediella* sp. in the zooplankton feeder group. However, its $\delta^{15}N$ value (highest value in Fig.3.4d) as well as its known predatory behaviour strongly indicates that there exists a trophic level between copepods and *Iphimediella* sp.

Fig.3.3. Carbon and Nitrogen isotopic ratios of 11 species of Antarctic amphipods: Wo—*Waldeckia obesa*, Ap—*Abyssorchomene plebs*, Eg—*Eurythenes gryllus*, Pc—*Pseudorchomene coatsi*, Es—*Epimeria similis*, Ege—*Epimeria georgiana*, Ep—*Eusirus perdentatus*, Ip—*Iphimediella* sp., Eh—*Echiniphimedia hodgsoni*, Ar—*Ampelisca richardsoni*, Df—*Djerboa furcipes*, spom—suspended particulate organic matter (data from Nyssen *et al.* 2002), Dm—brown macroalgae *Desmarestia mensiezii*.



As illustrated in Figure 3.3 where $\delta^{15}N$ is plotted against $\delta^{13}C$, the other iphimediid species, *E. hodgsoni*, topped the trophic food web together with *Iphimediella* sp. With a diet essentially composed of sponges (Dauby et al. 2001b, Nyssen unpublished results), the high trophic position of *E. hodgsoni* is unexpected. Stable isotope ratios of Antarctic sponges can be quite high (–22.3 and 12.5 ‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively (Nyssen et al. 2002). This may be due to assimilation of rapidly sedimenting and isotopically heavy aggregates of sea ice origin (Dunton 2001) or to assimilation of resuspended matter that was cycled repeatedly through the microbial loop (Hobson et al. 1995, Nyssen et al. 2002 and references therein). The fatty acid profile of *E. hodgsoni* did not show any sign of particular reliance on special food items. Its profile is dominated by 20:5(n-3) and 22:6(n-3) which are typical for marine organisms and predominant

in membrane lipids (Sargent & Whittle 1981, Sargent & Henderson 1986, Albers et al. 1996, Graeve et al. 2001).

In conclusion, our study demonstrates that both fatty acid composition and stable isotope ratios are suitable tools for trophic ecosystem analysis in their own right. Fatty acids point towards food web links and stable isotopes identify trophic positions. However, the use of only one of the two tools can lead to misinterpretations with serious implications. The combination of the two approaches creates a 2-dimensional biomarker assay with higher accuracy and better trophic resolution.

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