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Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods

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Abstract Antarctic amphipods from the Weddell Sea and Bransfield Strait were collected to investigate the impact of various species and feeding types on lipid and fatty acid compositions. In combination with digestive tract content analyses, such information can help clarify the type of feeding mode of the various amphipod species. Micro- and macropredatory amphipod species had only small amounts of triacylglycerols as storage lipids, whereas the deposit-feeder *Epimeria georgiana* was rich in triacylglycerols (55% of total lipids). The fatty acids 22:6(*n*-3), 20:5(*n*-3), 18:1(*n*-9) and 16:0 were major lipid components of most species. *Ampelisca richardsoni*, a suspension feeder, had a high amount of 18:4(*n*-3), a major component of cryptophytes and/or haptophytes, connected with feeding on sedimenting phytoplanktonic material and with a strong benthic-pelagic coupling. In *Oradarea edentata*, fragments of brown algae were found almost exclusively. The major fatty acid of the macroalgae, 20:4(*n*-6), replaced the 22:6(*n*-3) in the phospholipids and triacylglycerols of the amphipod. The sponge eater, *Echiniphimedia hodgsoni*, was rich in 16:1(*n*-7) and 18:1(*n*-7), suggesting that the unidentifiable organic matter was of diatom origin. *Eusirus perdentatus*, a typical predator, had high proportions of saturated and monounsaturated fatty acids, showing no specialisation in lipid and fatty acid composition. The fatty acid composition of *Epimeria georgiana* was similar to that of *Eusirus perdentatus*. However, high levels of triacylglycerols in *Epimeria georgiana* reflect periodical food plenty and starvation, due to its dependence on dead items. The fatty acid composition of the necrophage *Waldeckia obesa* was clearly different because of

the predominance of 18:1(*n*-9) (>40% of total fatty acids). This dominance is probably the result of feeding on highly degraded carrion-derived organic matter, which is the major food of *W. obesa*.

Introduction

In Antarctic benthic communities, the peracarid crustaceans and especially the Amphipoda are by far the most species-rich group and probably the most diversified with respect to lifestyles, trophic types, habitats and size spectra (De Broyer and Jazdzewski 1996). Despite their low relative biomass these crustaceans, especially the amphipods, constitute a significant group in terms of energy flux in the high-Antarctic shelf ecosystem. They are an important food source for many Antarctic demersal and benthic fishes (e.g. Olaso et al. 2000). A qualitative and quantitative characterisation of the trophic roles of amphipods and peracarids in general, at the level of the key species and of the whole taxocoenoses, could contribute significantly to a more accurate image of the trophic structure and fluxes in Antarctic benthic communities (Dauby et al., in press).

The ecofunctional and trophodynamic roles of Antarctic amphipods are still poorly known, despite the pioneering studies of Richardson (1977), Oliver and Slattery (1985), Slattery and Oliver (1986), Coleman (1989a, b, c, 1990a, b) and Klages and Gutt (1990a, b). The trophic types have been studied in less than 10% of amphipod species, with very few quantitative approaches so far (Dauby et al. 2001). For example, for the most important super-families of Antarctic amphipods (namely Eusiroidea and Lysianassoidea), the feeding type cannot often be deduced with certainty from the morphology of the feeding appendages.

The importance of lipid and fatty acid compositions as trophic markers has been demonstrated in various studies (Sargent and Whittle 1981; Graeve 1993; Graeve et al. 1994, 1997; Phleger et al. 1998). The advantage of

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applying lipid compositions to feeding studies is that trophic lipid markers integrate longer time periods than conventional methods. However, there is a lack of information on lipid compositions of Antarctic benthic amphipods. Most of the available literature covers lipid data of pelagic amphipods (e.g. Reinhardt and Van Vleet 1986; Hagen 1988; Phleger et al. 1998, 1999, 2000; Nelson et al. 2001). In our study, lipid and fatty acid compositions of various benthic amphipod species were analysed and compared with food preferences determined by digestive tract content analyses. The benefits of combining both methods to reveal feeding behaviour and food sources is highlighted.

Materials and methods

The benthic amphipods *Oradarea edentata*, *Ampelisca richardsoni*, *Echiniphimedia hodgsoni*, *Eusirus perdentatus*, *Epimeria georgiana* and *Waldeckia obesa* were caught in the southeastern Weddell Sea and the Antarctic Peninsula during the EASIZ II cruise with RV Polarstern (ANT XV/3, 13 January to 26 March 1998; Arntz and Gutt 1999). The animals were taken from various depths by different gear: box corer, Agassiz trawl, bottom trawl and TV grab. The different sampling sites are shown in Fig. 1. Immediately after sampling, individuals were sorted into species. Digestive tract content analyses were performed on specimens immediately preserved in 4% formaldehyde after sampling, or on freshly killed individuals. For observation of the feeding behaviour, specimens were kept for several days in aquaria (for details refer to Dauby et al. 2001, in press). The individuals dedicated to lipid analyses were transferred into glass vials containing dichloromethane:methanol 2:1 (v:v) and stored at -30°C until later analyses in the home laboratory.

Digestive tract analyses

Amphipods (17 individuals of *O. edentata*, 20 of *A. richardsoni*, 20 of *Echiniphimedia hodgsoni*, 19 of *Eusirus perdentatus*, 31 of *Epimeria georgiana* and 30 of *W. obesa*) were dissected under the dissecting microscope (Leica MZ12), using forceps and scissors. The digestive tract was cut at the oesophagus level and extracted together with midgut glands from the body. The digestive tract was separated from the midgut glands, opened and the contents spread on a microscope slide. Different dyes (Serva blue G, Fuchsin,

Bengal pink) were added depending on the material to be detected. The whole slide surface was examined with a compound microscope (Leitz Diaplan) equipped with a reflection contrast system. Some digestive contents (or parts of them) were also metallised in order to be more closely examined by SEM techniques.

The amount of food in digestive tracts was coded using arbitrary scores (from 1 to 4). Every item present in the digestive tract was determined to the lowest possible taxonomic group, and their proportions coded using a semi-quantitative coefficient based on the "percentage points" method (Hynes 1950; Williams 1981). A detailed description of the method is given in Dauby et al. (2001, in press). In addition, observations of feeding and general behaviour were performed on species kept in aquaria at -1°C (see Dauby et al. 2001, in press).

Lipid analyses

Twoxone adult animal of each species (max. size known: *O. edentata*, 11 mm, *A. richardsoni*, 33 mm, *Echiniphimedia hodgsoni*, 40 mm, *Eusirus perdentatus*, 87 mm *Epimeria georgiana*, 40 mm, *W. obesa*, 35 mm) were homogenised and total lipid extracted with dichloromethane:methanol (2:1; v:v) according to Folch et al. (1957). The lipid class compositions were determined according to Fraser et al. (1985) by thin-layer chromatography-flame ionisation detection (TLC-FID) with an IATROSCAN Mark IV TH 10. Different standard mixtures of marine lipids were used for identification (Hagen 1988): wax esters derived from copepod oil, triacylglycerols derived from fish oil and commercial standards [cholesterol, oleic acid, phosphatidylcholine (Sigma)]. An aliquot of the extract was used to separate the lipid classes by preparative thin-layer chromatography with silica gel 60H (Merck) on 20×20 cm glass plates. The bands were scraped off after visualising with a solution of 0.1% dichlorofluorescein in methanol, eluted in dichloromethane, evaporated to dryness and redissolved in hexane.

For the gas liquid chromatographic analysis of the fatty acids, aliquots of the total lipid extract and lipid classes were taken. Methyl esters of fatty acids were prepared by transesterification with 3% concentrated sulphuric acid in methanol for 4 h at 80°C . After extraction with hexane, their composition was determined with a Chrompack 9000 gas liquid chromatograph on a capillary column ($30 \text{ m} \times 0.25 \text{ mm}$; film thickness $0.25 \mu\text{m}$; liquid phase DB-FFAP), using temperature programming according to the method of Kattner and Fricke (1986). Fatty acids were identified by comparison of the retention time with those of standard mixtures. If necessary, identification was confirmed by converting fatty acids into their DMOX derivatives (Fay and Richli 1991), which were analysed with a Hewlett-Packard GC-MSD System (Waldbronn) fitted with a capillary column similar to that already described.

Results

Digestive tract contents

The results of the digestive tract content analyses are presented in Fig. 2. *O. edentata* was feeding on macroalgae since pieces of macroalgae, probably *Desmarestia* sp. (Phaeophyta), were found exclusively in its digestive tract. The digestive tract of *A. richardsoni* contained many phytoplankton (diatom) fragments (54%) embedded in unidentifiable organic matter (36%) forming a kind of acellular mucus without structure. Other items were generally small and/or fragmented, partially sponge spicules (7%). Some crustacean remains (3%) were also identified. The digestive tract contents of *Echiniphimedia hodgsoni* consisted mainly of spicules of sponges (61%). The remainder was phytoplankton-

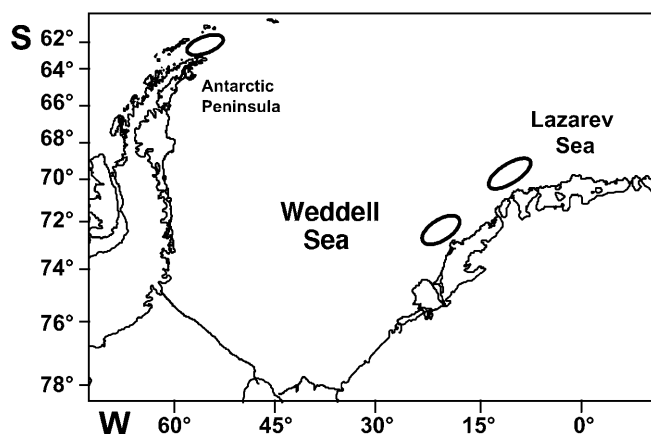
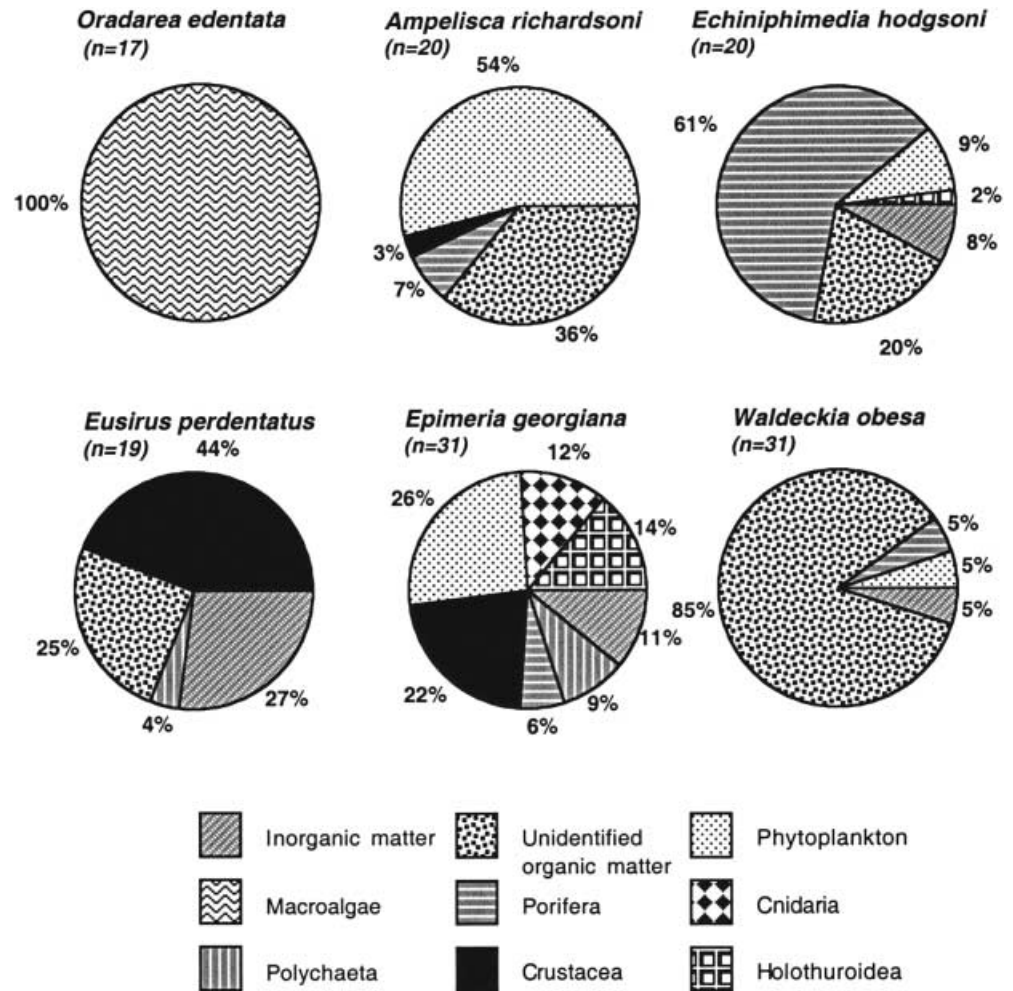


Fig. 1 Map and sampling locations of amphipods in Antarctica

Fig. 2 Digestive tract content of amphipods. Distribution of individual food items as percentage of the total content (*n* number of studied animals)



derived (9%), Holothuroidea (2%), unidentifiable organic matter (20%) and some sediment grains (8%). *Eusirus perdentatus* contained mostly crustacean remains (44%), unidentified organic matter (25%), mineral particles (27%) and some polychaete setae (4%) in its digestive tract. Items found in the digestive tract of *Epimeria georgiana* were very heterogeneous. The analyses exhibited remains of Crustacea (22%), polychaetes (9%), Holothuroidea (14%), Cnidaria (12%) and phytoplanktonic items (26%). Sponge spicules (6%) and mineral particles (11%) completed the food. The digestive tract of *W. obesa* was almost exclusively filled with carrion-derived organic matter (85%) originating from fishes, seabirds, cephalopods and benthic invertebrates. Other food items were plankton, inorganic matter and spicules, together composing 15% of the digestive tract contents.

Lipid and fatty acid composition

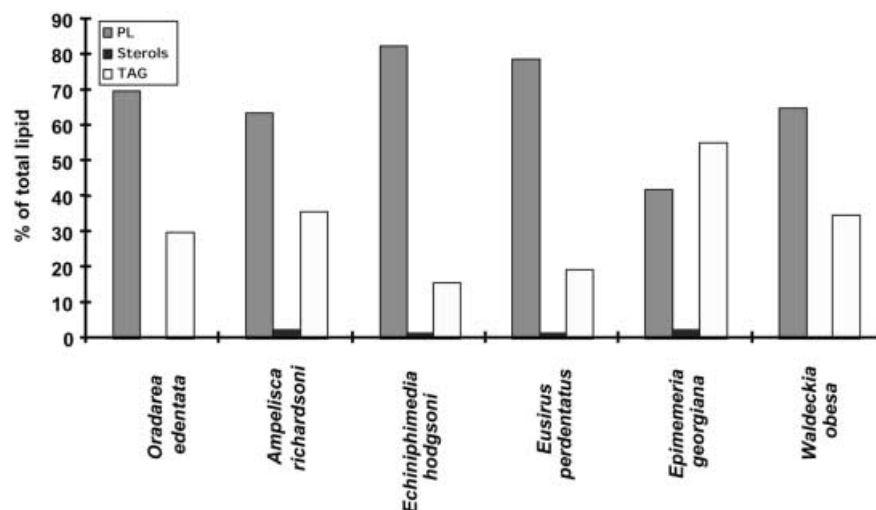
The lipid class and fatty acid compositions of total lipid extracts, triacylglycerols and phospholipids of amphipods revealed clear species-dependent differences. Major

lipid classes were phospholipids and triacylglycerols. Sterols and other lipids occurred only in trace amounts (less than 1%). Free fatty acids were not detected (Fig. 3).

In *O. edentata*, phospholipids and triacylglycerols accounted for 70% and 30% of total lipids, respectively (Fig. 3). One of the major fatty acids was 20:4(*n*-6), accounting for up to 20% of total fatty acids (Fig. 4). This fatty acid occurred in moderate amounts in *Epimeria georgiana* and only in trace amounts in the other amphipod species. Another major difference was the near absence of 22:6(*n*-3) in *O. edentata*. Other dominant fatty acids were 16:0, 18:1(*n*-9) and 20:5(*n*-3). Especially high quantities of 20:4(*n*-6) and 20:5(*n*-3) were found in the phospholipids, composing about 40% of the total. Moderate amounts (11%) of C₁₆ and C₁₈ polyunsaturated fatty acids were detected in all lipid fractions (Fig. 4).

A. richardsoni had a higher level of triacylglycerols (39%) than most of the other species (Fig. 3). The high amount of 18:4(*n*-3) in all lipid fractions, especially in the triacylglycerols (25%), was striking. Other major fatty acids were 20:5(*n*-3), 22:6(*n*-3), 16:0 and 16:1(*n*-7). The proportions of 20:5(*n*-3) and 22:6(*n*-3) in the

Fig. 3 Lipid class composition of amphipods as percentage of total lipid; PL: phospholipids, TAG: triacylglycerols



phospholipids (22% and 15%, respectively) were slightly higher than in the triacylglycerols (Fig. 4). The ratio of 18:1(*n*-9)/18:1(*n*-7) was low (2.6).

The lipids of the sponge eater *Echiniphimedia hodgsoni* consisted mostly of phospholipids (82%) and small amounts of triacylglycerols (16%) (Fig. 3). The fatty acid composition of total lipids was dominated by 16:0, 16:1(*n*-7), 18:1(*n*-9), 18:1(*n*-7) and 20:5(*n*-3) (Fig. 4). The proportions of polyunsaturated fatty acids with 16 and 18 carbon atoms were relatively low. 20:5(*n*-3) was dominant in the triacylglycerols, whereas 22:6(*n*-3) was detected in trace amounts in this lipid class. The phospholipids of *Echiniphimedia hodgsoni* showed high levels of 16:0, 18:1(*n*-9), 20:5(*n*-3) and 22:6(*n*-3). The elevated level of 18:1(*n*-7) in all lipid fractions resulted in a low 18:1(*n*-9)/(*n*-7) ratio (1.9).

The total lipids of *Eusirus perdentatus* were dominated by phospholipids (79%) (Fig. 3). The fatty acids 16:0, 18:1(*n*-9), 20:5(*n*-3) and 22:6(*n*-3) were the major components in the total lipids and lipid classes (Fig. 5). The two polyunsaturated fatty acids had especially high proportions in the phospholipids. In the triacylglycerol fraction, 16:1(*n*-7) and 18:1(*n*-7) were slightly higher, composing 6% and 7%, respectively.

Epimeria georgiana exhibited the largest portion of triacylglycerols of all amphipods, with 55% of the total lipids (Fig. 3). The fatty acid compositions were dominated by 16:0, 18:1(*n*-9), 20:5(*n*-3) and 22:6(*n*-3). Other important fatty acids were 16:1(*n*-7), 18:1(*n*-7) and 20:4(*n*-6). In the phospholipids, 16:0, 20:4(*n*-6), 20:5(*n*-3) and 22:6(*n*-3) occurred in higher proportions than in the triacylglycerols (Fig. 5).

W. obesa was also rich in triacylglycerols (37%) (Fig. 3). Its fatty acid composition differed from all other species since 18:1(*n*-9) made up almost 50% of total fatty acids (Fig. 5). The fatty acid compositions of the triacylglycerols and phospholipids were very similar to that of the total lipids. Slightly enhanced levels of 20:5(*n*-3) and 22:6(*n*-3) were found in the phospholipids. *W. obesa* had the highest 18:1(*n*-9)/(*n*-7) ratio of all

amphipods investigated, with 18.6 for triacylglycerols and 17.1 for phospholipids.

Discussion

Determination of lipid content and composition can improve interpretation of trophic interactions and pathways of organic material in the water column, as shown by Graeve et al. (1994) and Cripps and Hill (1998) for marine zooplankton. The trophic types of the species investigated here are described as herbivorous feeder (*O. edentata*), suspension feeder (*A. richardsoni*), micropredator (*Echiniphimedia hodgsoni*), macropredator (*Eusirus perdentatus*), deposit-feeder (*Epimeria georgiana*) and necrophage (*W. obesa*) (Dauby et al., in press; Y. Scailteur, unpublished work). The lipid and fatty acid compositions of these Antarctic amphipods were characterised by distinct differences partially reflecting their different food preferences. High levels of storage lipids in only one species and moderate levels in two other species show that reliance on lipid reserves in the amphipods was generally low. This is in accordance with results reported for Arctic benthic amphipods (Graeve et al. 1997) and benthic amphipods from cold shallow waters (Kawashima et al. 1999).

Due to the usual dominance of phospholipids, the polyunsaturated fatty acids were abundant in most species, since they are typical for marine organisms and predominate among the membrane lipids (Sargent and Whittle 1981; Sargent and Henderson 1986; Albers et al. 1996). These polyunsaturates are normally 20:5(*n*-3) and 22:6(*n*-3) in marine animals; however, in *O. edentata* 22:6(*n*-3) was absent and, in contrast, elevated levels of 20:4(*n*-6) were observed. Although in some species large amounts of the diet were of planktonic origin, we found no evidence for incorporation of long-chain monounsaturated fatty acids (20:1 and 22:1) from zooplankton such as herbivorous calanoid copepods. So far, this incorporation has only been observed for the lysianassoid

Fig. 4 Fatty acid composition of the total lipid, triacylglycerols (TAG) and phospholipids (PL) of *Oradarea edentata*, *Ampelisca richardsoni* and *Echiniphimedia hodgsoni* as mass percent of total fatty acids

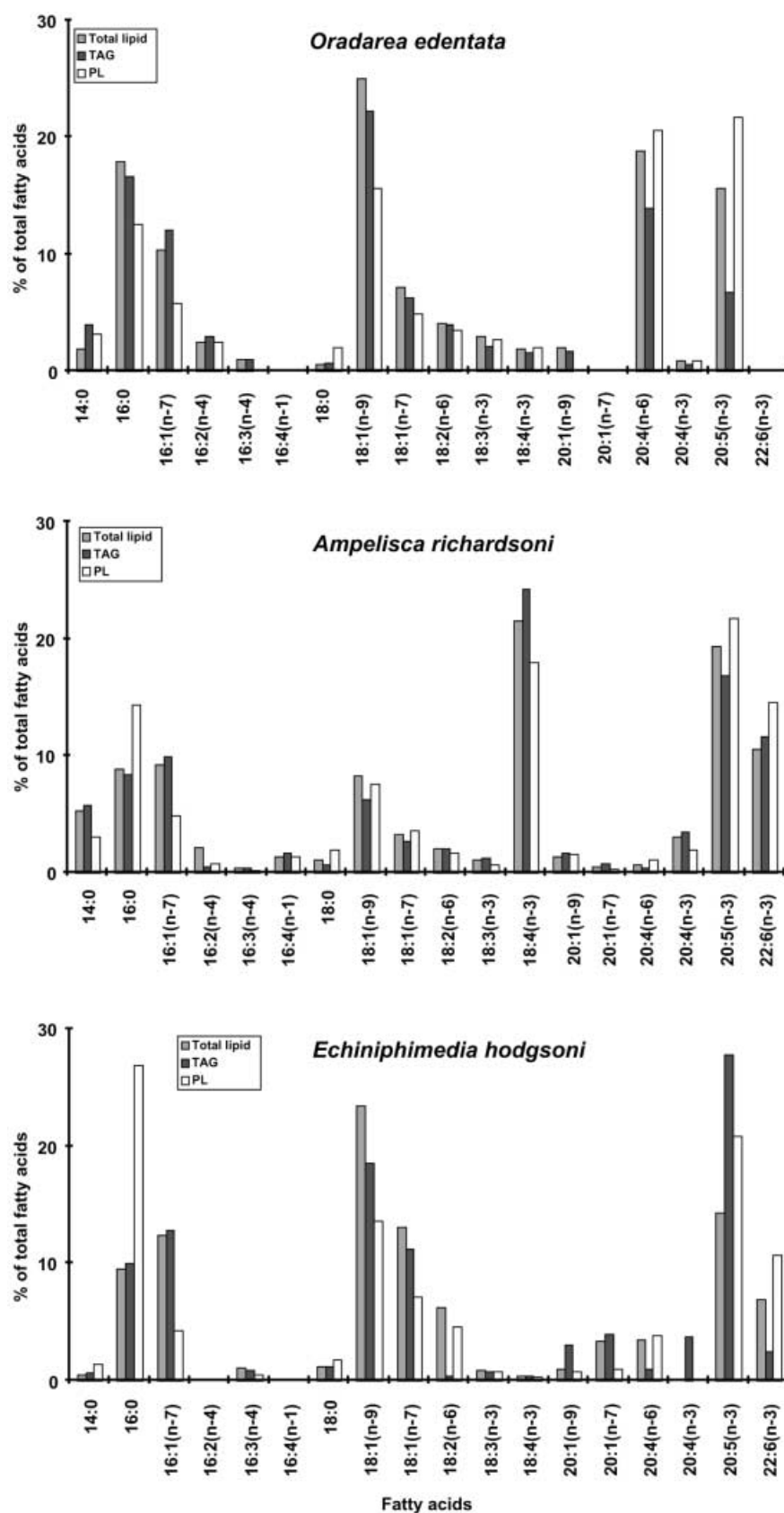
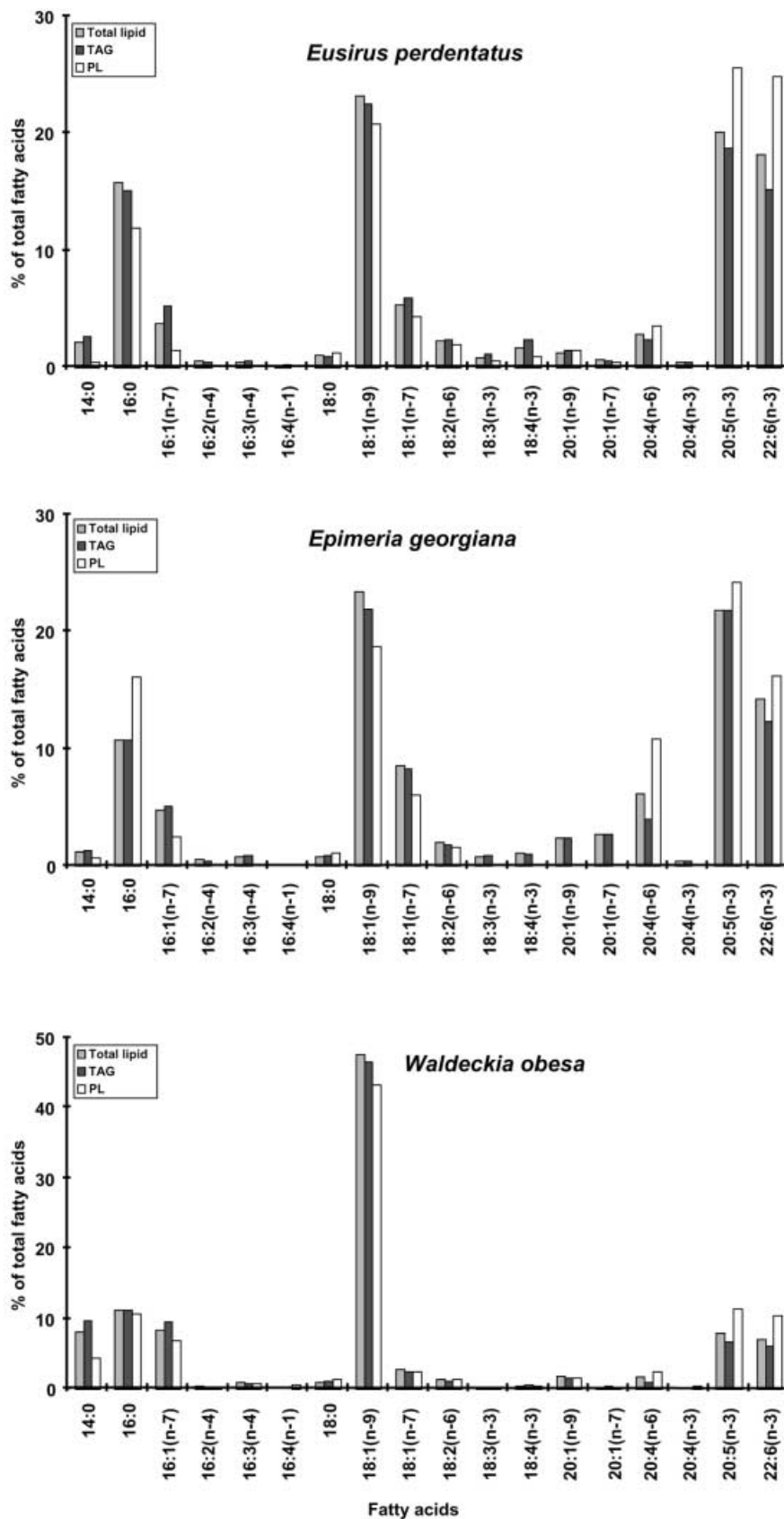


Fig. 5 Fatty acid composition of the total lipid, triacylglycerols and phospholipids of *Eusirus perdentatus*, *Epimeria georgiana* and *Waldeckia obesa* as mass percent of total fatty acids



amphipod, *Anonyx nugax*, from the Arctic (Graeve et al. 1997). Also wax esters, the major storage lipids in most zooplankton species (e.g. Kattner and Hagen 1995), were not found in the Antarctic benthic amphipods, but small amounts were detected in the hyperiid amphipod, *Primno macropa* (Nelson et al. 2001). Thus, if amphipods are feeding on wax ester-rich organisms, these dietary lipids are not incorporated or utilised for metabolic requirements.

Among the investigated amphipods, the sublittoral herbivore *O. edentata* differs markedly from the other species examined in this and other studies due to its predominant feeding on macroalgae (Y. Scailteur, unpublished work). *O. edentata* contained only pieces of macroalgae, probably *Desmarestia* sp., in its digestive tract. Moderate amounts of triacylglycerols in the amphipod suggest that food is available year round. The most remarkable feature of this species is the high level of 20:4(*n*-6). This fatty acid certainly originates from macroalgae (M. Graeve, unpublished work) and is incorporated unmodified in the lipids of the amphipod. 20:5(*n*-3) is another major fatty acid in macroalgae but 22:6(*n*-3) occurs only in trace amounts (Fleurence et al. 1994; Khotimchenko 1995). 20:4(*n*-6) has obviously replaced the 22:6(*n*-3) not only in the depot lipids but also in the phospholipids where it probably becomes part of the membrane structure. It is known that (*n*-6) polyunsaturated fatty acids originate from benthic algae and some of the benthic invertebrates including molluscs, coelenterates, echinoderms and crustaceans contain substantial quantities of (*n*-6) polyunsaturated fatty acids, especially 20:4(*n*-6) (Sargent and Whittle 1981; Fleurence et al. 1994; Khotimchenko 1995; M. Graeve, unpublished work). In microalgae, especially *Porphyrium* sp., Ackman et al. (1968) also found considerable amounts of 20:4(*n*-6), accounting for up to 25% of total fatty acids.

The epibenthic suspension feeder *A. richardsoni* is dependent on material sinking from the upper water column (Dauby et al. 2001). L.D. Gerdes (personal communication) found a large number of *A. richardsoni* specimens in corer samples from bottoms scoured by icebergs (mainly between 150 and 350 m). This species may be an early coloniser of new soft substrates, feeding on organic material sedimenting rapidly to the bottom in shallow waters. The amount of triacylglycerols it contains reflects a moderate reliance on storage lipids, which may enable this species to survive periods of starvation. The digestive tract content of *A. richardsoni* was rich in diatom residues within organic material without any structure. The ingestion of diatoms is supported by the diatom marker fatty acids, 16:1(*n*-7) and 20:5(*n*-3) (Kates and Volcani 1966; Ackman et al. 1968; Graeve et al. 1994). However, the dominant fatty acid 18:4(*n*-3) points to the organic matter being largely of non-diatomous origin in which the diatom shells were embedded. Digestive tract analysis cannot differentiate the structureless mucus of organic matter, but 18:4(*n*-3) reflects feeding on material originating from phytoplankton,

such as cryptophytes and/or haptophytes (Harrington et al. 1970; Nichols et al. 1991; Graeve 1993; Graeve et al. 1994; Swadling et al. 2000). 18:4(*n*-3) might be the signature of feeding on a decaying *Phaeocystis* bloom although 18:4(*n*-3) is not usually dominant in Antarctic *Phaeocystis* (Swadling et al. 2000). Those blooms are often observed in the Southern Ocean, and efficient sedimentation of *Phaeocystis*-derived organic matter has been described (Riebesell 1993; Wassmann 1994; Lancelot et al. 1998). Colonies or solitary cells of the haptophyte *Phaeocystis pouchetii* are ingested by a wide range of marine invertebrates (Weisse et al. 1994). The combination of digestive tract and fatty acid analyses reveals a strong benthic-pelagic coupling, indicating that *A. richardsoni* feeds mostly on phytoplankton-derived material. However, it must be remembered that blooms are short-term events and that the resulting fatty acid signature may vary with the dominating phytoplankton species.

Echiniphimedia hodgsoni is a large micropredator which principally feeds on sponge material. Feeding on sponges is possible due to the functional morphology of its mouth parts. With a special cutting mechanism, *Echiniphimedia hodgsoni* is able to bite out tough sponge material (Coleman 1989a). Since sponges are very common on the Antarctic shelf, this species does not rely on storage lipids, as reflected by its low triacylglycerol content. In sponges a wide range of unusual fatty acids has been described, including even-chain, odd-chain and branched-chain fatty acids, as well as very long-chain fatty acids (Carballeira and Reyes 1990; Dembitsky et al. 1994; Carballeira and Shalabi 1995). These unusual fatty acids were not detected in *Echiniphimedia hodgsoni*. However, the remaining digestive tract content, which could only be described as unidentifiable organic matter, may be assigned to a diatom origin on the basis of the considerable amounts of 16:1(*n*-7) and 18:1(*n*-7) in the animal. These fatty acids are indicative of diatom-derived particulate organic matter, which may originate directly from diatoms or from prey organisms feeding on diatoms. This is also supported by a higher 20:5/22:6 ratio. Slightly higher levels of 20:1 fatty acids may suggest feeding on copepods (Graeve et al. 1994; Phleger et al. 1998).

In contrast to these amphipods, *Eusirus perdentatus* is a macropredatory carnivorous species, which was observed feeding mainly on living crustaceans, polychaetes and other mobile invertebrates (Klages and Gutt 1990a). Although *Eusirus perdentatus* is a passive sit-and-wait predator, it seems to catch or find sufficient prey to be almost independent of lipid deposits, as shown by its relatively small proportions of triacylglycerols compared to the other species. In consequence, the typical membrane polyunsaturated fatty acids were dominant. This species shows no specialisation either in lipid or fatty acid compositions, which demonstrates a strongly opportunistic feeding behaviour.

The fatty acid composition of *Epimeria georgiana* is very similar to that of *Eusirus perdentatus*, although it

prefers dead instead of living food items (Dauby et al. 2001). *Epimeria georgiana* is also a weakly mobile deposit-feeder. The heterogeneous digestive tract contents (fragments of crustaceans, polychaetes and cnidarians, but also diatoms and radiolarians) reflect a very diverse feeding behaviour. The ever-present sand grains confirm deposit-feeding. Compared to the other amphipods, *Epimeria georgiana* was rich in triacylglycerols, which may enable this species to compensate for longer periods of food scarcity. The reliance on dead food items may result in longer periods of starvation compared to predatory species. The fatty acid composition of *Epimeria georgiana* shows no clear indication of a special feeding behaviour. The high level of 18:1(*n*-9) points to feeding on degraded material, such as crustacean remains; however, the small proportion of phytoplanktonic marker fatty acids present indicates feeding on material of planktonic origin.

W. obesa has the most obligate scavenging lifestyle of the amphipods studied (Arnaud 1970). It is a large amphipod with a wide circum-Antarctic distribution, occurring in subsurface waters and down to 900 m depth in the Weddell Sea (De Broyer and Klages 1990; De Broyer and Jazdzewski 1993). The main fraction of its digestive tract content was carrion-derived organic matter, which comes from necrophagous feeding on prey such as fish and invertebrate carcasses, and probably also on seal or penguin carrion (Dauby et al., in press). The exact share of the source cannot be precisely defined. The relatively high amount of triacylglycerols in the lipids indicates periods of starvation, where storage lipids may be important for survival. This finding is supported by feeding experiments that revealed that *W. obesa* is well adapted to long periods of starvation (Chapelle et al. 1994). Coleman (1991) reported that this species can endure periods of up to 18 months without feeding. The fatty acid composition of *W. obesa* is unique, since it is dominated to a great extent by 18:1(*n*-9), which occurred in similar amounts of nearly 50% in phospholipids and triacylglycerols. Lipid-rich fish are potential food items, being known to contain high levels of 18:1(*n*-9) (Hagen et al. 2000) but not as high as found for *W. obesa*. It is noteworthy that the phospholipids were also dominated by 18:1(*n*-9), since phospholipids are normally considered to require high amounts of polyunsaturates for maintenance of membrane fluidity. There is also no evidence of an increased bacterial activity, since odd-chain, branched and hydroxy fatty acids were not detected. The small amount of additional organic material in the digestive tract of *W. obesa* might be influenced by diatomous components. However, due to the predominance of 18:1(*n*-9), no indication of food of phyto- or zooplanktonic origin can be obtained.

Conclusions

Lipids integrate feeding behaviour over long periods of time, whereas the digestive tract content showed us the

recent food uptake. Combining the two methods, the amphipod species studied exhibited a lipid and digestive tract content pattern indicative of their typical life strategies and trophic niches. These are reflected by specific fatty acid compositions and food items, as shown for *O. edentata* feeding on macroalgae and for *A. richardsoni*, where fatty acid composition is probably dependent on sedimentation events of periodically occurring phytoplankton blooms. Conversely, less specific fatty acid compositions indicate opportunistic feeding on living or dead food items with similar fatty acid compositions. It is remarkable how amphipods can adapt their fatty acid composition to the nutritional conditions, not only in the storage lipids but also in the membrane lipids. The differences between the fatty acid compositions of the phospholipids as structural components of membranes and the triacylglycerols as storage lipids are small. We therefore propose that further investigations of trophic linkages of benthic amphipods should be done with analysis of the total lipid fatty acid composition. Future lipid investigations should consider reproductive strategies and seasonal variations, since there are clear indications that amphipods can shift their food items depending on season and food availability. Additional feeding experiments and analysis of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values could help clarify food-web interactions of benthic amphipods.

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