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Lipids of Antarctic salps and their commensal hyperiid amphipods

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Abstract Antarctic salps (*Salpa thompsoni* and *Ihlea racovitzai*) and their commensal hyperiid amphipods (*Vibilia antarctica*, *Cyllopus lucasii* and *C. magellanicus*) were collected near Elephant Island, in the South Shetland Islands, during 1997 and the salp-rich year 1998. The sterol composition of aggregate *S. thompsoni* and *I. racovitzai* (mostly 24-methyl-5,22*E*-dien-3β-ol, 24-nordehydrocholesterol, cholesterol and *trans*-dehydrocholesterol) was reflected in the sterol composition of the commensal amphipods and was consistent with a herbivorous planktonic diet. This was not the case for solitary *S. thompsoni*, with 24-methylenecholesterol as the major sterol. There was a greater abundance of aggregate salp stanols in 1997 (11.7% total sterols) than 1998 (5.2%) and these different stanol levels were reflected in the commensal amphipods. Eicosapentaenoic acid [20:5(*n*-3)] and docosahexaenoic acid [22:6(*n*-3)] were the major polyunsaturated fatty acids (PUFA) in all organisms. Octadecapentaenoic acid [18:5(*n*-3)] comprised 0.4–5.8% (of total fatty acids) in all 1998 salps and amphipods, but was absent in 1997 samples. This suggests a greater presence of dinoflagellates or other species rich in 18:5(*n*-3) in the "salp year" 1998. Very long chain PUFA (C_{24}, C_{26}, C_{28}) were also only detected in 1998 samples (up to 5.3%), reflecting commensalism and greater presence of dinoflagellates or species containing very long chain PUFA. Examination of the biomarker lipids has provided an indication of trophic interactions for these Antarctic salps and their commensal hyperiid amphipods.

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Introduction

Salpa thompsoni is the most abundant salp of the Southern Ocean, with the subtropical convergence as its northern limit. The salp *Ihlea racovitzai* is less abundant and is found in high-latitude Antarctic water (Casareto and Nemoto 1986). The hyperiid amphipods *Cyllopus lucasii*, *C. magellanicus*, and *Vibilia antarctica* are ectocommensals on *S. thompsoni* (Madin and Harbison 1977; Torres et al. 1994a,b; Loeb et al. 1998). Most *S. thompsoni* are aggregate chain-forming organisms, which sexually reproduce (92% of total population, 1997 and 1998; Loeb et al. 1997, 1998); the remainder are solitary individuals which asexually produce the aggregate salps, sometimes resulting in massive blooms (Foxton 1966). High salp densities have occurred in the Antarctic Marine Living Resources (AMLR) survey area near Elephant Island during 1983–1984, 1988–1990, 1992–1994, and 1998 (Loeb et al. 1997, 1998). In 1998, *S. thompsoni* was the numerically dominant zooplankton species (56–75% relative abundance of all zooplankton), followed by post-larval *Thysanoessa macrura*, copepods, post-larval *Euphausia superba*, and *I. racovitzai*. 1998, therefore qualified as a "salp year." Salp years appear to correlate with the 4–5 year period of the Antarctic Circumpolar wave, which propagates changes in sea surface temperatures and wind stress direction (White and Peterson 1996). During 1997, a "transition year," copepods were numerically dominant, followed by *S. thompsoni*, post-larval *T. macrura*, *E. frigida* and post-larval *E. superba*.

Salps are reported to have very high rates of feeding, and are indiscriminate feeders competing with krill for phytoplankton and even consuming large numbers of slow-swimming krill larvae (Huntley et al. 1989). The role of predation upon salps is not clear, although cod,

sardine, and tuna consume temperate salps (Silver 1975) and albatross have been reported to consume Antarctic salps (Foxton 1966).

Salps are gelatinous urochordates, with 95% water and less than 1% lipid, as percent wet weight (Hagen 1988). Only a limited number of studies to date have examined the lipid composition of Antarctic salps. Lipid classes of *S. thompsoni* include mostly polar lipid (42%) and triacylglycerols (TAG, 55%) (Reinhardt and Van Vleet 1986). Other lipid classes include wax esters, sterol esters, free fatty acids, hydrocarbons, sterols, and glyceryl ethers (Reinhardt and Van Vleet 1986; Kolakowska 1987; Hagen 1988). Antarctic hyperiid amphipods have more lipid than salps (*V. propinqua*, 2% lipid as percent wet weight, and *C. lucasii*, 6% lipid), which is primarily TAG, with less polar lipid (Hagen 1988). A preliminary analysis of the sterols and fatty acids of *S. thompsoni* was performed as part of a larger trophodynamic study (Phleger et al. 1998).

The purpose of this paper is to examine specific sterols and fatty acid biomarkers of the salps *S. thompsoni* (aggregate and solitary stages) and *I. racovitzai*, and their hyperiid amphipod commensals, *V. antarctica*, *C. lucasii*, and *C. magellanicus*. The lipid signatures of these animals are used to help clarify the commensal relationship between salps and hyperiid amphipods, and also to compare a "salp year" (1998) and a "transition year" (1997). Salps play a major role in Antarctic marine zooplankton ecosystems, competing with krill for food resources, and a careful evaluation of their trophodynamics by biomarker lipids can contribute to providing a basis for understanding Antarctic food chains.

Materials and methods

Sample description

Salps and amphipods were collected by Isaacs-Kidd Midwater Trawl fitted with a 505-μm-mesh plankton net from the R/V *Yuzhmorgeologiya* during January and February 1997 and 1998. The samples were obtained as part of the AMLR Field Study conducted annually in the Elephant Island region of the Antarctic Peninsula (Loeb et al. 1997; Martin 1997, 1998). The AMLR study area is located between 60–62.5°S and 53–59°W. The net was obliquely towed to 170-m depth for about 30 min at a speed of 2 knots, or to 10 m above the bottom in shallower waters. Samples were frozen in liquid nitrogen as soon as possible after sorting on board ship. They were then transported frozen (dry ice) by air to CSIRO Marine Research, in Hobart, Tasmania, where they were maintained at −70 °C prior to analysis. Salp fresh weights were 13 g for *S. thompsoni* (solitary), 0.4–4 g for *S. thompsoni* (aggregate) and 0.2–0.4 g for *I. racovitzai*. Commensal amphipod fresh weights were 0.06–0.14 g for *V. antarctica*, 0.05–0.15 g for *C. lucasii*, and 0.04–0.09 g for *C. magellanicus*. The 1997 *S. thompsoni*, sample represents five pooled individuals. The 1998 *I. racovitzai* samples represent two pooled individuals each; two of the *V. antarctica* samples were pooled (two each), and one *C. lucasii* sample includes two pooled individuals.

Lipid extraction

Samples were quantitatively extracted using a modified Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2:1:0.8 v/v/v); the sample was extracted overnight and the phases were separated the following day by the addition of chloroform and water (final solvent ratio 1:1:0.9 v/v/v methanol:chloroform:water). The total solvent extract was concentrated (i.e. solvents removed in vacuo) using rotary evaporation at 40 °C. Lipid class analyses were generally conducted immediately; samples were stored for no more than 3 days in a known volume of chloroform.

Lipid classes

An aliquot of the total solvent extract was analyzed using an Iatroscan MK V TH10 thin-layer chromatography-flame-ionization detector (TLC-FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman and Nichols 1991). Samples were applied in duplicate or triplicate to silica gel SIII chromarods (5 μm particle size) using 1 μl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane/diethyl ether/acetic acid (60:17:0.2 v/v/v), a mobile phase resolving non-polar compounds such as wax esters (WE), triacylglycerols, free fatty acids (FFA), and sterols (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 v/v) was also used to separate hydrocarbon from WE and TAG from diacylglyceryl ether (DAGE). After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, triglyceride derived from a commercial fish oil product (Max EPA), and DAGE purified from shark liver oil; 0.1–10 μg range]. A laboratory standard of WE derived from orange roughy oil was used for peak identification, and steryl ester was used for quantification of WE. WE and steryl esters coelute in the systems used. Based on the TLC-FID analyses and subsequent analysis of component fatty acids and alcohols by gas chromatography (GC), steryl esters were either absent or only present as trace components. Peaks were quantified on an IBM-compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% (Volkman and Nichols 1991).

An aliquot of the total solvent extract was treated with potassium hydroxide in methanol (5% w/v) and saponified under nitrogen for 3 h at 80 °C. Non-saponifiable neutral lipids (e.g., sterols, glyceryl ether diols and hydrocarbons) were extracted into hexane/chloroform (4:1 v/v, 3 × 1.5 ml) and transferred to sample vials. Following acidification of the remaining aqueous layer using hydrochloric acid (pH = 2), fatty acids were extracted and methylated to produce their corresponding fatty acid methyl esters using methanol/hydrochloric acid/chloroform (10:1:1 v/v/v; 80 °C, 2 h). Products were extracted into hexane/chloroform (4:1 v/v, 3 × 1.5 ml) and stored at −20 °C. The non-saponifiable neutral lipid fractions were treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 60 °C, 1 h) to convert sterols to their corresponding TMSi (trimethylsilyl) ethers.

Gas chromatographic (GC) analyses of methyl esters of fatty acids were performed with a Hewlett Packard 5890A GC (Avondale, Pa.) equipped with an HP-1 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50 °C. After 1 min, the oven temperature was raised to 150 °C at 30 °C min^{-1}, then to 250 °C at 2 °C min^{-1} and finally to 300 °C at 5 °C min^{-1}. Peaks were quantified with either DAPA Scientific or Waters Millenium software (Milford, Mass.). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component abundance.

GC-mass spectrometric (GC-MS) analyses were performed on a Fisons MD 800 GC-mass spectrometer (Manchester, UK) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

Determination of double bond configuration in fatty acids

Dimethyl disulphide (DMDS) adducts of monounsaturated fatty acids were formed by treating the total fatty acid methyl esters of selected samples with dimethyl disulfide (Dunkelblum et al. 1985; Nichols et al. 1986a). Adducts were then extracted using hexane/chloroform (4:1 v/v) and treated with BSTFA to form TMS derivatives prior to GC-MS analysis.

Results

Lipid classes

Polar lipids comprise the major lipid class in both salps (62–93% of total lipid) and amphipods (57–78%, Table 1). Triacylglycerols were the second most abundant lipid class in *S. thompsoni* aggregate form (A) (19%) and the salp *I. racovitzai* (33%), but comprise only 1.5% in *S. thompsoni* solitary form (S) (Table 1). TAG were also the second most abundant lipid class in the amphipods with higher values in *C. lucasii* (40%) than in *C. magellanicus* (20%) and *V. antarctica* (22%). Sterols accounted for 1.7–4.8% of total lipids in all organisms. Minor amounts of wax esters (0.1–0.4%) occurred in all amphipods, whereas no WE were detected in salps. Free fatty acids were low in all samples (0.0–2.0%). Total lipid was higher in the amphipods *C. lucasii* (23.8 mg g^{-1} wet weight) and *V. antarctica* (13.6 mg g^{-1}) whereas it was lower in *C. magellanicus* (2.2 mg g^{-1}), *I. racovitzai* (3.4 mg g^{-1}) and least in *S. thompsoni* (A) (0.7 mg g^{-1}) and *S. thompsoni* (S) (0.3 mg g^{-1}) (Table 1).

Sterols

The major sterols in *S. thompsoni* (A) (1998) included 24-methyl-5,22*E*-dien-3β-ol (32.9% of total sterols), 24-nordehydrocholesterol (15.8%), cholesterol (12.9%), and *trans*-dehydrocholesterol (12.4%) (Table 2). There were lesser percentages of *cis*-dehydrocholesterol/27-nor-24-methylcholest-5,22*E*-dien-3β-ol (5.7% of total sterols), 24-methyl-5α-cholest-22*E*-en-3β-ol (5.2%), 24-methylenecholesterol (4.8%), and 24-ethylcholesterol (6.0%). The sterol composition of *I. racovitzai* was similar to that of 1998 *S. thompsoni* (A) and included 31.7% 24-methyl-5,22*E*-dien-3β-ol, 12.8% 24-nordehydrocholesterol, 15.6% cholesterol, and 10.2% *trans*-dehydrocholesterol (Table 2). The percentages of minor sterols in *I. racovitzai* were also similar to *S. thompsoni* (A) and included 5.5% *cis*-dehydrocholesterol/27-nor-24-methylcholest-5,22*E*-dien-3β-ol, 2.5% 24-methyl-5α-cholest-22*E*-en-3β-ol, 6.2% 24-methylenecholesterol, and 7.0% 24-ethylcholesterol. The solitary salp, *S. thompsoni* (S) differed from 1998 *S. thompsoni* (A) and *I. racovitzai* in that its major sterols were 52.3% 24-methylenecholesterol and 13.8% 24-methyl-5,22*E*-dien-3β-ol. *S. thompsoni* (S) also contained the sterols 24-nordehydrocholesterol (4.6%) and *cis*-dehydrocholesterol/27-nor-24-methylcholest-5,22*E*-dien-3β-ol (7.2%), as well as some other minor sterols (Table 2). Total stanols in 1998 *S. thompsoni* (S) were 10.5% and included 2.1% 24-methyl-5α-cholest-22*E*-en-3β-ol, 1.2% 24-nordehydrocholestanol, and 7.2% dehydrocholestanol (Table 2). The sterol composition of *S. thompsoni* (A) (1997) was similar to that of the 1998 *S. thompsoni* (A); however, many of the major sterols were lower in abundance; there was 22.9% 24-methyl-5,22*E*-dien-3β-ol, 9.5% nordehydrocholesterol, 7.5% cholesterol and 6.9% *trans*-dehydrocholesterol (Table 2). In contrast, 1997 *S. thompsoni* (A) had more 24-methylenecholesterol (12%) and 24-ethylcholesterol (4.5%). There was a total of 11.7% stanols in 1997 *S. thompsoni* (A) including 24-nordehydrocholestanol (2.0%), dehydrocholestanol (1.9%), cholestanol (1.1%), 24-methylenecholesta-nol (1.4%), and 5.3% 24-methyl-5a-cholest-22*E*-en-3β-ol (Table 2).

The major sterols in the hyperiid amphipods were similar to those in the aggregate salps but had slightly different proportions. 24-Nordehydrocholesterol was present in *V. antarctica*, *C. lucasii*, and *C. magellanicus* at 8.7–26.5% of total sterols (Table 2). The 1998 values for 24-nordehydrocholesterol were 17.5–26.5%, whereas 1997 results included two lower values (8.7% in *C. magellanicus*, 9.9% in *C. lucasii*) and 21.4% in *V. antarctica*. *Trans*-dehydrocholesterol was 15.7–26.2% in all amphipods, with slightly lesser percentages in 1998 amphipods (15.7–22.7%) than 1997 amphipods (24.2–26.2%). 24-Methyl-5,22*E*-dien-3β-ol (13.0–17.8%) and cholesterol (12.7–20.0%) were also major amphipod sterols (Table 2). There were lower levels of *cis*-dehydrocholesterol/27-nor-24-methylcholest-5,22*E*-dien-3β-ol

Table 1 Percentage lipid class composition of 1998 Antartic salps and commensal amphipods (presented as mean ± SD) (*TAG* triacyglycerol, *A* aggregate, *S* solitary)

	Wax esters	TAG	Free fatty acids	Sterols	Polar lipids	Lipids as mg g^{-1} wet weight
Salpa thompsoni (A) (n = 4)	–	19.1 ± 7.0	2.0 ± 0.4	2.7 ± 0.6	76.2 ± 7.8	0.7 ± 0.2
S. thompsoni (S)	–	1.5	1.0	4.8	92.7	0.3
Ihlea racovitzai (n = 3)	–	33.4 ± 9.2	1.8 ± 0.6	2.8 ± 0.5	62.0 ± 10.0	3.4 ± 1.2
Cyllopus lucasii (n = 4)	0.1 ± 0.2	40.1 ± 19.2	0.3 ± 0.3	2.3 ± 1.0	57.3 ± 18.2	23.8 ± 13.9
C. magellanicus (n = 3)	0.3 ± 0.4	19.5 ± 2.3	0.3 ± 0.3	1.7 ± 0.5	78.3 ± 2.0	2.2 ± 1.7
Vibilia antarctica (n = 3)	0.4 ± 0.1	22.4 ± 11.8	–	2.5 ± 0.5	74.7 ± 11.4	13.6 ± 4.5

Table 2 Percentage sterol composition of 1997 and 1998 Antarctic salps and commensal amphipods (presented as mean ± SD) (*A* aggregate, *S* solitary)

	1997				1998					
	Salpa thompsoni A[a]	*Cyllopus lucasii* ($n = 2$)	*Cyllopus magellanicus* ($n = 2$)	*Vibilia antarctica* ($n = 3$)	*Salpa thompsoni* A ($n = 4$)	*Salpa thompsoni* S	*Ihlea racovitzai* ($n = 3$)	*Cyllopus lucasii* ($n = 3$)	*Cyllopus magellanicus* ($n = 3$)	*Vibilia antarctica* ($n = 3$)
Sterol										
24-Nordehydrocholesterol	9.5	9.9 ± 0.5	8.7 ± 0.9	21.4 ± 1.5	15.8 ± 2.0	4.6	12.8 ± 0.6	21.2 ± 1.4	17.5 ± 3.2	26.5 ± 3.6
24-Nordehydrocholesterol	2.0	0.3 ± 0.5	0.4 ± 0.5	1.6 ± 1.4	–	1.2	–	1.8 ± 0.3	1.3 ± 0.7	2.8 ± 0.5
cis-Dehydrocholesterol/27-Nor-24-methylcholest-5,22*E* -dien-3B-ol	5.7	2.1 ± 3.0	3.1 ± 0.3	9.3 ± 0.8	5.7 ± 0.7	7.2	5.5 ± 0.5	4.8 ± 1.4	4.1 ± 1.0	3.8 ± 0.8
trans-Dehydrocholesterol	6.9	26.2 ± 2.2	24.2 ± 0.3	15.6 ± 3.4	12.4 ± 1.2	1.0	10.2 ± 2.6	15.7 ± 1.6	16.8 ± 3.6	22.7 ± 1.9
Dehydrocholestanol	1.9	1.0 ± 1.4	1.9 ± 1.1	0.9 ± 0.8	–	7.2	–	1.0 ± 0.9	0.5 ± 0.9	–
Cholesterol	7.6	18.5 ± 2.6	20.0 ± 0.3	14.6 ± 0.7	12.9 ± 5.1	1.2	15.6 ± 1.4	17.5 ± 2.9	12.7 ± 1.0	16.8 ± 3.0
Cholestanol	1.1	1.9 ± 2.7	2.2 ± 0.6	1.2 ± 0.2	–	–	–	1.9 ± 0.4	2.0 ± 0.8	0.3 ± 0.5
Desmosterol	4.3	4.8 ± 1.4	5.8 ± 0.4	5.2 ± 0.8	–	–	–	0.4 ± 0.7	1.2 ± 1.0	–
24-Methylcholest-5,22*E*-dien-3B-ol	22.9	17.8 ± 0.2	17.2 ± 0.2	14.2 ± 0.9	32.9 ± 2.3	13.8	31.7 ± 1.4	16.2 ± 0.3	13.0 ± 2.1	15.2 ± 2.3
24-Methyl-5a-cholest-22*E*-en-3B-ol	5.3	2.1 ± 3.0	2.7 ± 0.5	1.3 ± 1.7	5.2 ± 3.6	2.1	2.5 ± 2.1	1.8 ± 1.3	2.6 ± 1.3	0.9 ± 0.8
24-Methylenecholesterol	12.0	14.7 ± 4.5	13.1 ± 1.0	7.7 ± 1.5	4.8 ± 0.4	52.3	6.2 ± 1.1	6.2 ± 1.1	7.2 ± 1.3	2.7 ± 1.0
24-Methylenecholestanol	1.4	0.3 ± 0.4	0.4 ± 0.6	0.7 ± 0.7	–	–	–	–	–	–
24-Ethylcholest-5,22*E*-dien-3B-ol	2.9	–	–	0.2 ± 0.3	2.1 ± 2.4	2.1	–	–	–	–
24-Ethylcholesterol	4.5	0.1 ± 0.1	–	1.8 ± 0.6	6.0 ± 0.7	3.4	7.0 ± 0.7	1.8 ± 0.4	6.7 ± 9.4	2.6 ± 1.1
Isofucostanol	7.1	0.2 ± 0.3	0.3 ± 0.4	3.9 ± 1.3	–	–	–	–	–	–
24-Ethyl-5a-cholest-7-en3B-ol	0.3	–	–	–	–	–	–	–	–	–
4-Methyl-24-ethyl-cholesta-5,24(28)-dien-3B-ol	4.4	0.1 ± 0.1	–	0.3 ± 0.5	–	–	–	–	–	–
Other	0.2	–	–	0.1	2.2 ± 2.6	3.9	8.5 ± 1.2	9.7 ± 3.7	14.4 ± 4.1	5.7 ± 2.5

[a] Five pooled individuals

(2.1–9.3% of total sterols), and 24-methylenecholesterol (2.7–14.7%). Levels of 24-methylenecholesterol were greater in 1997 amphipods (7.7–14.7%) than in 1998 amphipods (2.7–7.2%). Total stanols in all amphipods were 3.8–5.7% of total sterols (Table 2), and included 24-nordehydrocholestanol (0.3–2.8%), dehydrocholestanol (0.0–1.9%), cholestanol (0.3–2.2%), 24-methyl-5α-cholest-22*E*-en-3β-ol (0.9–2.7%), and 24-methylenecholestanol (0.4–0.7%, only in 1997 amphipods; 0.0% in 1998). Dinosterol was not detected in either salps or amphipods.

Fatty acids

Eicosapentaenoic acid [EPA, 20:5(*n*-3)] and docosahexaenoic acid [DHA, 22:6(*n*-3)] were the two major polyunsaturated fatty acids (PUFA) (18.1–24.1% and 11.0–17.4% of total fatty acids, respectively) in all samples (Table 3). The PUFA 18:5, not detected in 1997 samples, comprised 0.4–5.8% in all 1998 samples of salps and amphipods. The lowest level (0.4% of total fatty acids) of 18:5 occurred in *S. thompsoni* (S) and the highest (5.8%) in the salp *I. racovitzai*. The PUFA 18:4 comprised 3.7–15.9% in all samples from each year in both salps and amphipods.

The very long chain (VLC) PUFA C_{24}, C_{26}, and C_{28} were not detected in 1997 samples, but comprised 4.9% in 1998 *S. thompsoni* (S) (C_{24} and C_{26} PUFA only), 5.3% in *V. antarctica* (C_{24} and C_{26} PUFA), and 3.2% in *C. lucasii* (Table 3). The C_{24} and C_{26} VLC-PUFA comprised four to six components of each chain length, respectively. All components showed mass spectra characteristic of PUFA, with base peaks at *m*/*z* 79 and other prominent ions at *m*/*z* 67, 91, 105, 119, and 131 with *m*/*z* 74 generally at 10–15% of the base peak abundance. When present, the C_{28} VLC-PUFA was in lower abundance than the C_{24} and C_{26} VLC-PUFA; only one C_{28} component was observed. C_{28} PUFA was detected in 1998 aggregate *S. thompsoni* and *I. racovitzai* (0.1% of total fatty acids, both species), but was not detected in solitary *S. thompsoni*. There were lower levels of VLC-PUFA in *C. magellanicus* (0.0–0.3% C_{24} and C_{26}) and C_{28} PUFA (0.1%) was the only long chain PUFA in *S. thompsoni* (A) and (S). There was also 0.1% C_{28} PUFA in *C. lucasii* and *C. magellanicus*. Total PUFA in all samples were 50.3–62.5% of total fatty acids (Table 3).

Total monounsaturated fatty acids (MUFA) varied from 12.2 to 18.3% in all salps and from 13.7 to 18.7% in amphipods. These MUFA included primarily 16:1(*n*-7)c (2.9–9.0% of total fatty acids), 18:1(*n*-9)c (1.9–6.7%), and 18:1(*n*-7)c (1.1–7.2%). The principal saturated fatty acids in all samples were 16:0 (13.0–20.9% of total fatty acids), 14:0 (2.8–11.3%), 18:0(0.6–1.4%), and 15:0 (0.6–2.6%) (Table 3).

Discussion

Lipid classes

Lipid class percentages were similar for the hyperiid amphipods, the aggregate salp (*S. thompsoni*) and the salp *I. racovitzai* (Table 1). Polar lipid, the major lipid class, is primarily a structural component of membranes, whereas TAG, the second most abundant lipid class, is a short-term energy reserve lipid. Total lipid was higher in two amphipod species (*C. lucasii* and *V. antarctica*) than in all salps; this may reflect stored energy for future winter conditions when salp hosts are not available beneath pack ice. *C. lucasii* sampled within pack ice had less lipid than those from open water (Torres et al. 1994a); *C. lucasii* and *V. stebbingi* were reported to also have lower metabolic rates during winter (Torres et al. 1994b). The low lipid content, together with the low proportion of storage lipid in solitary *S. thompsoni* (1998), is consistent with a lower nutritional status for this stage. Asexual reproduction in solitary salps may also be related to low TAG, whereas high TAG in aggregate salps may be required for sexual reproduction. The low level of WE in amphipods (not present in salps) may reflect feeding on foods other than salps, such as copepods or their larvae, which are known to be rich in WE. The low level of WE also suggests that this dietary contribution is only minor.

Sterols

The sterol composition of aggregate salps and *I. racovitzai* is similar to the three species of ectocommensal hyperiid amphipods (Table 2), and reflects a broad diversity of sterols, which is consistent with a herbivorous diet. The similar sterol spectra of aggregate salps and the amphipods support a possible salp-amphipod food chain. Species of *Vibilia* feed primarily on the food strand of the salps and may eat parts of the salp itself (Madin and Harbison 1977). Sterols are useful indicators of dietary composition in marine herbivores (Virtue et al. 1993). The sterol composition of aggregate *S. thompsoni* here is in general agreement with published data for this species (Mimura et al. 1986), although our levels for 24-methyl-5,22*E*-dien-3β-ol (23–33%) are somewhat greater than the 18% (as brassicasterol) reported by Mimura et al. (1986). We confirmed the presence of desmosterol in *S. thompsoni* (1997 animals only), first reported by Mimura et al. (1986) (Table 2). The sterol composition of the solitary salp (over 50% 24-methylenecholesterol) is quite different from the amphipods. These findings are consistent with the amphipods analyzed in this study being commensals on aggregate *S. thompsoni*. Surface particles, the diet of North Atlantic Ocean *S. fusiformis*, were assimilated into the animal as evidenced by the similar sterol

Table 3 Percentage fatty acid composition of 1997 and 1998 Antarctic salps and commensal amphipods (presented as mean ± SD) (*A* aggregate, *S* solitary). *Other* includes all components present at < 1%: 12:0, 13:0, 17:0, 20:0, 22:0, 24:0, il4:0, il5:0, al5:0, il6:0, il7:0, al7:0, il9:0, 4,8,12-TMTD, 14:1(*n*-7)c, 14:1(*n*-5)c, 16:1(*n*-9)c, 18:1(*n*-7)t, 20:1(*n*-9)c, 20:1(*n*-7)c, 22:1(*n*-1)c, 22:1(*n*-9)c, 22:1(*n*-7)c, 18:3(*n*-6), C_{19}PUFA, 20:3(*n*-6), 20:2, 22:5(*n*-3), 22:2(*n*-6), C_{28}PUFA

	1997				1998					
	Salpa thompsoni A[a]	*Cyllopus lucasii* (*n* = 2)	*Cyllopus magellanicus* (*n* = 2)	*Vibilia antarctica* (*n* = 3)	*Salpa thompsoni* A (*n* = 4)	*Salpa thompsoni* S	*Ihlea racovitzai* (*n* = 3)	*Cyllopus lucasii* (*n* = 3)	*Cyllopus magellanicus* (*n* = 3)	*Vibilia antarctica* (*n* = 3)
Fatty acid										
14:0	3.7	5.5 ± 1.1	5.5 ± 1.6	2.8 ± 0.5	4.8 ± 1.0	7.1	11.3 ± 1.0	4.6 ± 1.1	4.5 ± 1.8	3.3 ± 0.1
15:0	0.6	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.4	1.4 ± 0.2	2.6	1.1 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
16:0	20.9	16.0 ± 2.3	13.1 ± 2.8	14.1 ± 3.1	13.7 ± 1.0	17.9	13.0 ± 0.7	14.9 ± 2.5	13.5 ± 0.5	16.2 ± 1.6
18:0	0.8	1.4 ± 0.2	1.2 ± 0.3	1.1 ± 0.3	0.8 ± 0.1	1.1	0.6 ± 0.0	1.2 ± 0.5	1.0 ± 0.3	0.9 ± 0.2
Sum saturates	26.0	23.6 ± 3.8	20.4 ± 4.8	18.7 ± 4.3	20.7 ± 2.3	28.7	26.0 ± 1.8	21.7 ± 4.2	19.6 ± 2.7	21.3 ± 2.0
16:1(*n*-7)c	2.9	8.6 ± 0.8	9.0 ± 2.6	4.7 ± 0.6	4.2 ± 0.2	4.2	7.5 ± 0.3	5.4 ± 0.9	5.8 ± 1.8	5.2 ± 0.7
16:1(*n*-7)t	0.1	–	–	0.1 ± 0.1	1.0 ± 0.2	0.5	–	–	–	–
16:1(*n*-5)c	0.4	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.1	1.1 ± 0.2	0.5	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.3	0.9 ± 0.1
18:1	–	–	–	–	0.5 ± 0.0	0.6	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
18:1(*n*-9)c	6.7	5.7 ± 0.2	5.1 ± 0.1	5.3 ± 0.6	2.0 ± 0.5	2.1	1.9 ± 0.0	3.4 ± 0.3	4.3 ± 0.8	5.7 ± 0.3
18:1(*n*-7)c	7.2	2.1 ± 0.2	1.6 ± 0.1	2.0 ± 0.4	3.2 ± 0.5	4.6	1.1 ± 0.1	1.6 ± 0.4	1.5 ± 0.5	2.1 ± 0.5
18:1(*n*-5)	0.4	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.0	0.5 ± 0.1	0.6	0.5 ± 0.0	0.8 ± 0.2	1.0 ± 0.3	1.0 ± 0.1
19:1	0.4	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.3	0.6 ± 0.1	1.0 ± 0.3	0.3 ± 0.6	0.7 ± 0.1
20:1(*n*-11/13)	0.2	0.5 ± 0.1	0.3 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	–	–	1.2 ± 0.1	–	0.5 ± 0.2
Sum monounsaturates	18.3	18.7 ± 1.8	17.5 ± 3.3	15.0 ± 2.1	13.0 ± 1.9	13.4	12.2 ± 0.6	14.3 ± 2.4	13.7 ± 4.4	16.6 ± 2.1
C_{16}PUFA	0.5	1.4 ± 0.0	2.5 ± 0.1	2.6 ± 0.4	2.7 ± 0.2	0.6	5.1 ± 0.7	2.9 ± 0.7	1.8 ± 0.2	1.2 ± 0.3
18:5(*n*-3)	–	–	–	–	1.1 ± 0.2	0.4	5.8 ± 0.1	2.5 ± 1.1	1.7 ± 0.7	1.7 ± 0.6
18:4(*n*-3)	3.7	8.3 ± 1.9	9.3 ± 2.1	15.9 ± 3.0	13.5 ± 1.3	4.5	9.2 ± 0.3	10.8 ± 3.1	11.7 ± 0.4	8.8 ± 1.8
18:2(*n*-6)	4.0	2.8 ± 0.0	3.0 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	1.8	2.9 ± 0.1	2.6 ± 0.8	2.4 ± 0.4	2.5 ± 0.3
18:3(*n*-3)	1.2	0.5 ± 0.1	0.6 ± 0.1	1.9 ± 0.3	2.7 ± 0.2	1.1	2.2 ± 0.0	1.7 ± 0.5	1.3 ± 0.3	1.3 ± 0.3
20:5(*n*-3)	24.2	22.6 ± 1.6	24.1 ± 4.3	21.9 ± 0.3	21.5 ± 1.9	19.9	21.5 ± 1.2	18.1 ± 0.7	21.9 ± 3.4	19.1 ± 0.8
20:4(*n*-3)	0.5	0.7 ± 0.1	0.9 ± 0.1	1.4 ± 0.4	0.5 ± 0.1	0.3	0.4 ± 0.0	1.0 ± 0.1	1.2 ± 0.1	0.8 ± 0.1
20:2(*n*-6)	0.5	1.2 ± 0.3	0.9 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.5	0.2 ± 0.0	0.9 ± 0.1	1.4 ± 0.1	0.5 ± 0.0
C_{21}PUFA	0.5	0.5 ± 0.1	0.6 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.7	–	0.7 ± 0.1	1.1 ± 0.4	0.6 ± 0.0
22:6(*n*-3)	17.4	15.6 ± 4.3	15.6 ± 5.8	14.7 ± 2.8	15.9 ± 1.9	15.6	11.0 ± 0.6	13.1 ± 0.1	14.4 ± 3.5	15.0 ± 1.1
C_{24}PUFA	–	–	–	–	–	2.7	–	2.0 ± 1.7	–	3.0 ± 0.7
C_{26}PUFA	–	–	–	–	–	2.2	–	1.2 ± 1.2	0.3 ± 0.3	2.3 ± 0.5
Sum polyunsaturates	52.2	53.6 ± 8.4	57.5 ± 12.9	62.5 ± 7.6	61.7 ± 6.1	50.3	58.3 ± 3.0	57.5 ± 10.2	59.2 ± 9.8	56.8 ± 6.5
Other	3.2	4.1	4.6	3.8	4.6	7.6	3.5	6.5	7.5	5.3

[a] Five pooled individuals

composition (Laureillard et al. 1989). The 24-methyl-5,22*E*-dien-3β-ol in the Antarctic salps and amphipods in the present study may have originated from dietary prymnesiophytes, such as *Phaeocystis*, and diatoms where brassicasterol is a major sterol (Nichols et al. 1991; Tsitsa-Tzardis et al. 1993). The sterol 24-methylenecholesterol is the main sterol in the diatom *Chaetoceros* (Tsitsa-Tzardis et al. 1993). *Trans*-dehydrocholesterol is an intermediate in cholesterol synthesis, and was reported in Antarctic jellies (*Calycopsis borchgrevinki*, *Beroe cucumis*, *Arctopodema ampla*, *Periphylla periphylla*, 11–23%), as well as the hyperiid amphipod *Themisto gaudichaudii* (15%), which eats jellies (Phleger et al. 1998). Dinosterol, a biomarker for dinoflagellates, was not detected in either the salps or amphipods. This finding suggests that either dinoflagellates are absent from the diet or, if members of this algal group are present, they are species that do not contain dinosterol.

Salp stanols were also reflected in the commensal hyperiid amphipods. 24-Methyl-5α-cholest-22*E*-en-3β-ol in aggregate salps was 5% for 1997 and 1998, and the amphipods had 1–3% 24-methyl-5α-cholest-22*E*-en-3β-ol for both years. Annual differences in salp stanols also appeared in the amphipods. Four stanols other than 24-methyl-5α-cholest-22*E*-en-3β-ol, totaling 6.5%, occurred in 1997 aggregate salps. In contrast, these stanols were not detected in 1998 salps (Table 2). Relative to the salps, lower levels of stanols were detected in the 1997 amphipods, with none detected in the 1998 samples of this species. The presence of stanols reflect diet; for example, in the marine dinoflagellate *Scrippsiella* sp., cholestanol composed 24% of total sterols (Mansour et al. 1999), and a species of *Gymnodinium* isolated from Australian waters contained 24% dinostanol (Nichols et al. 1984). Stanols could also be markers for detrital material. The fact that stanols were reflected in 1997 commensal amphipods, we believe, is indicative of a dietary origin.

Fatty acids

Eicosapentaenoic acid [20:5(*n*-3)] and docosahexaenoic acid [22:6(*n*-3)] levels observed in this study are in agreement with Mimura et al. (1986) and Phleger et al. (1998) for *S. thompsoni*. These fatty acids are also the major PUFA in the commensal amphipods (Table 3). These PUFA are obtained primarily from the diet, because marine zooplankton lack the ability to synthesize EPA and DHA from precursor fatty acids.

The unusual octadecapentaenoic acid 18:5(*n*-3) is synthesized by some species of dinoflagellates from 20:5(*n*-3) (Joseph 1975). It was first reported in marine zooplankton (copepods and chaetognaths) by Mayzaud et al. (1976), who recognized its usefulness as a biomarker. We detected 18:5(*n*-3) in all our 1998 samples of salps and amphipods, but not in 1997 (Table 3) or 1996 (Phleger et al. 1998). This suggests a greater presence of either dinoflagellates or some other species containing 18:5(*n*-3) in the AMLR study area in 1998. Although algal composition studies are not available for the AMLR study area in 1997 and 1998, major changes have been reported in the zooplankton composition by Loeb et al. (1998). The 1998 zooplankton were dominated by *S. thompsoni* (56–75% relative abundance), whereas the 1997 zooplankton were dominated by copepods, with *S. thompsoni* ranking second (Loeb et al. 1998). The ranking of species by percentage composition (of the total zooplankton population) between 1997 and 1998 also differed substantially as outlined in the Introduction.

Similar to the finding with 18:5(*n*-3), VLC-PUFA were also only detected in our 1998 salps and amphipods, but not in the 1997 animals. The VLC-PUFA octacosaheptaenoic acid [28:7(*n*-6)] and octacosaoctaenoic acid [28:8(*n*-3)] have been recently identified in seven dinoflagellate species including *Prorocentrum mexicanum*, *Prorocentrum micans*, *Scrippsiella* sp., *Symbiodinium microadriaticune*, *Gymnodinium* sp., *G. sanguineum*, and *Fragilidium* sp. (Mansour et al. 1999). VLC-PUFA usually do not exceed tenths of a percent of total fatty acids except in sponges (Rezanka 1989). Over 48% of the fatty acids in the marine sponge *Microciona prolifera* have C_{23}–C_{28} chain lengths, which appear to be biosynthesized by chain elongation (Morales and Litchfield 1977). Of this unusually high proportion of long-chain fatty acids, dienes accounted for 22%, trienes 14.2%, and other polyenes 0.2%. The remainder were long-chain MUFA. We found up to 5% (Table 3) and Mansour et al. (1999) found up to 2.2% in dinoflagellates. In addition, 4.3% 26:1, Δ17c, 2% 26:0, and 1.1% 26:1 were found in the Antarctic sea-ice diatom *Nitzschia cylindrus* (Nichols et al. 1986b). VLC-PUFA (C_{24}–C_{28}) have also recently been reported in three crustacean species of the Order Bathynellacea collected from Lake Baikal and the caves of Central Europe (Rezanka and Dembitsky 1999). The VLC-PUFA content reached almost 2% in the crustaceans. Differences in the distribution of these components between species were proposed to be due mainly to different environmental conditions, dietary habits and geographical spreading. Elevated amounts of saturated very long chain fatty acids (VLC-FA) occur in humans with x-linked adenoleukodystrophy and Zellweger's syndrome (Poulos 1989). Animal cell culture and intact studies have demonstrated that synthesis of 22:6(*n*-3) involves C_{24} PUFA (Ishihara et al. 1998; Innis et al. 1999).

The finding of VLC-PUFA in lower members of the marine food chain may have wider interest. With increasing recognition of the beneficial health attributes of marine omega-3 PUFA-containing oils, utilization of such oils in nutraceuticals and other products is predicted to increase. Detailed analytical data on the composition of marine oils together with appropriate toxicological testing will be needed. The occurrence of the VLC fatty acids, including VLC-PUFA, in the lower Antarctic food-web organisms, and also in other marine plants and animals (Morales and Litchfield 1977; Nichols et al. 1986b; Rezanka 1989; Mansour et al.

1999), suggests that further knowledge is required on the possible occurrence of these novel components in commercial fish and other marine oils (e.g. micro-algal-derived) now in use.

The VLC-PUFA (Table 3) in the 1998 salps and amphipods are, we believe, possible evidence for the greater importance of dinoflagellates or some other phytoplankton group, in the AMLR study area in 1998 versus 1997. VLC-PUFA were also not detected in *Salpa thompsoni* collected in 1996 from the same study area (Phleger et al. 1998). The C_{24} and C_{26} VLC-PUFA were detected only in the solitary form of *Salpa thompsoni*, while C_{28} VLC-PUFA were detected in 1998 aggregate *Salpa thompsoni* and *I. racovitzai*. Because the solitary *Salpa thompsoni* reproduces asexually and the aggregate form is the sexual phase, metabolic differences may account for activity rates of their chain elongation systems. Depending on which lipid class encompasses the VLC-PUFA (polar lipids in the sponge, *Microciona prolifera*; Jefferts et al. 1974), the transfer to amphipods could also indicate that the amphipods prey to some extent on salp tissue. A further mechanism to account for variations observed is that possible differences may occur in the retroconversion of dietary-derived C_{28} PUFA.

The PUFA 18:4(*n*-3) was detected in 1998 salps (9–14%) and amphipods (9–12%), 1997 salps (4%) and amphipods (8–16%) (Table 3), and 1996 salps (8%, Phleger et al. 1998). The PUFA 18:4(*n*-3) is a major fatty acid in the prymnesiophyte *Isochrysis* sp. (T-ISO) and in the cryptomonad *Chroomonus salina* (Volkman et al. 1989). We also found low levels of oleic acid, 18:1(*n*-7)c, 3–7% in 1997 and 1998 *Salpa thompsoni*, similar to levels (3%) detected in 1996 (Phleger et al. 1998). Lower levels of oleic acid in *Salpa thompsoni* reflect a phytoplankton-rich diet, consistent with observations noted above for the sterol profile of these animals.

In summary, two Antarctic salp species and three commensal hyperiid amphipod species have shown similar lipid compositional profiles. The exception was solitary *Salpa thompsoni* (1998), which was depleted in storage lipid. These profiles are consistent with a common dietary, largely phytoplanktonic, intake for all species. The finding of specific signature lipids, such as 18:5(*n*-3) and VLC-PUFA (C_{24}–C_{28}), in 1998 samples highlights interannual variation between the 1997 "transition year" and the 1998 "salp year" seasons; presently the source of these components remains to be determined. We recommend the analysis of both mixed community and, where possible, isolated phytoplankton species from these waters to further examine the sources for these unusual signature lipids.

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