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The lipid, fatty acid and fatty alcohol composition of the myctophid fish *Electrona antarctica*: high level of wax esters and food-chain implications

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Abstract: The myctophid, *Electrona antarctica*, was collected by trawl from the Elephant Island region of the Antarctic Peninsula, and from East Antarctica near 61°S and 93°W. Total lipid was higher in Elephant Island *E. antarctica* (whole fish, 466–585 mg g^{-1} dry weight) than those from Eastern Antarctica (394–459 mg g^{-1}). Wax esters comprised 86.2–90.5% of total lipid in *E. antarctica* flesh. There were no significant differences between Eastern Antarctica and Elephant Island in total wax ester levels, or in levels of wax esters between different tissues analysed. Oily bones (up to 326 mg g^{-1} in the neurocranium) characterized *E. antarctica* from both locations, with wax esters as the major skeletal lipid class (67.0–87.9%, percent of lipid). The wax esters may have a buoyancy role in *E. antarctica*. The only substantial amount of triacylglycerols (29.4%) were found in the viscera of Elephant Island fish. The principal fatty acids of all fish analysed included the monounsaturated fatty acids 18:1(n-9) and 16:1(n-7), with lower levels of 16:0 and 14:0 saturated acids. Fatty alcohols were dominated by the saturated 16:0 and 14:0 (37.8–47.8%) and the monounsaturated 18:1(n-9) and 18:1(n-7) (38.3–59.2%). The low ratio of 22:1/20:1 alcohols observed for *E. antarctica* is consistent with a diet of amphipods, copepods and other items low in 22:1 alcohols.

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Key words: *Electrona antarctica*, fatty acids, fatty alcohols, lipids, myctophid fish, wax esters

Introduction

The Myctophidae are an important cosmopolitan midwater fish family, including over 230 species (Wisner 1974, Neighbors 1988). In the Antarctic area, there are about 13 species, of which *Electrona antarctica*, *E. carlsbergii*, *Protomyctophum anderssoni* and *Gymnoscopelus nicholsi* are the most abundant (Sabourenkov 1991). The biomass of all mesopelagic myctophids south of 40°S has been recently estimated at between 70–396 x 10^6 tons (Sabourenkov 1991, Lubimova *et al.* 1983).

The presence of high levels of wax esters (82–91%, as percent of total lipid) in certain myctophid fishes was first recognized by Nevenzel *et al.* (1969) for *Lampanyctus ritteri*, *Stenobrachius leucopsarus*, and *Triphoturus mexicanus* collected from Hawaiian and Southern California Bight waters. Similar high wax ester levels have been found in other myctophids including *S. nannochir* and *L. regalis* (Saito & Murata 1996). According to Neighbors (1988), of the 41 myctophid species analysed for lipid, nine species contain greater percentages of wax esters than triacylglycerols. Reinhardt & Van Vleet (1986) first reported levels of wax esters in *E. antarctica* from Croker Passage in the Antarctic Penninsula as 45–62% of total lipid. Wax esters are important long-term energy reserve molecules (Lee & Patton 1989) and therefore have advantages over the more common lipid triacylglycerols, a short-term energy reserve molecule, during long periods of food deprivation such as occur during the Antarctic winter. Wax esters are also more buoyant than triacylglycerols, which may help midwater fishes such as myctophids maintain their position in the water column. They are essentially non-compressible, in contrast to swimbladder gases, and thus may make diurnal vertical migration a simpler task (Nevenzel *et al.* 1969).

Although as noted above, wax esters accounted for 45–62% of the lipid in *E. antarctica* from Antarctic Croker Passage waters, detailed quantitative lipid compositional data are not available for this species. The purpose of the present study, therefore, has been to compare the lipid composition of flesh, skull, spine, and viscera. Large deposits of oil have been found in the skeleton of other fish species, particularly the wax ester-rich orange roughy, *Hoplostethus atlanticus* (Phleger & Grigor 1990). A secondary objective was to compare lipids in fish from two different geographic locations in Antarctic waters namely, the Elephant Island area of Western Antarctica and the Eastern Antarctica area near 93°W longitude. Previous studies of *E. antarctica* have only examined animals from one location. In light of recent biomass estimates for myctophids, and the commercial potential of their oils (Nichols *et al.* 1994), we were also

particularly interested in the analysis of wax esters, including component fatty acids and fatty alcohols, using modern TLC-FID and GC-MS techniques.

Materials and methods

Sample description

The myctophid *Electrona antarctica* was collected by Isaacs-Kidd Midwater Trawl from the RV *Yuzhmorgeologia* on 1 and 2 February 1996, from Stations A-64 (61°S, 55°W) and A-71 (61°S, 54°W). These samples ($n = 3$) were obtained as part of the Antarctic Marine Life Research (AMLR) Field Study conducted annually in the Elephant Island region of the Antarctic Peninsula (Martin 1996, Hewitt & Demer 1991). In both cases the net was towed to 170 m depth for about 30 min. Fish were frozen at -10°C as soon as possible after sorting and counting on board ship. They were then transported frozen by air to CSIRO Oceanography, Hobart, Tasmania, where they were maintained at -60°C prior to analysis. *E. antarctica* ($n = 3$) was also collected by rectangular midwater trawl (to 195 m) from the RV *Aurora Australis* on "Expedition Broke" during February and March 1996, from East Antarctica (Station 57; 61°50.26'S, 93°32.31'W). They were frozen in liquid nitrogen immediately and transported frozen to CSIRO Marine Research, Hobart, for analysis.

Each fish was thawed before dissection. The neurocranium and vertebral centra of each fish were dissected, rigorously cleaned to remove flesh and neural tissue, and chopped into fine pieces with a razor prior to lipid extraction. The viscera was dissected from each fish, and included the gut and the gut contents. Fishes from the Elephant Island study area ranged in standard length from 96–100 mm and fresh weight from 13.81–16.54 g. Fishes from East Antarctica ranged in standard length from 57–72 mm and fresh weight from 4.24–4.84 g.

Lipid extraction

Samples were quantitatively extracted using a modified Bligh & Dyer (1959) one-phase methanol: chloroform: water extraction (2:1:0.8 v/v/v); samples were 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 (TSE) was concentrated (i.e. solvents removed in vacuo) using rotary evaporation at 30°C. All samples were made up to a known volume in chloroform and stored at -20°C. Samples were stored for up to three days; lipid analysis was conducted immediately.

Lipid classes

An aliquot of the TSE was analysed using an Iatroscan MK V TH10 TLC-FID analyser to determine the abundances of individual lipid classes (Volkman & Nichols 1991). Each sample of the same fish was 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 was hexane-diethyl ether-acetic acid (60:17:0.2 v/v), a mobile phase resolving non-polar compounds such as wax esters, sterol esters, and triacylglycerols, free fatty acids and sterols. A second non-polar solvent system of hexane-diethyl ether (96:4 v/v) was also used for all samples to resolve hydrocarbons, wax esters, triacylglycerols and diacylglyceryl ethers. After development, the chromarods were oven dried and analysed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, triolein and diacylglyceryl ether, the latter purified from shark liver oil; 0.1–10 μg range). Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% (Volkman & Nichols 1991).

A one-factor analysis of variance was performed for the lipid class data between samples collected from the two different sites and between lipid classes of each tissue analysed. Duplicate and triplicate samples are from lipid extracts from tissues of different fish. Fisher's PLSD (protected least significant difference) multi-comparison test was applied and the results reported use a percentage data used for statistical analysis.

Fatty acids and fatty alcohols

An aliquot of the TSE was treated with methanol-hydrochloric acid-chloroform under nitrogen (10:1:1 v/v/v; 80°C, 2 h) to form fatty acid methyl esters (FAME) and fatty alcohols. Following the addition of water, products were extracted into hexane/chloroform (4:1 v/v, 3 x 1.5 ml), transferred to vials, reduced under a stream of nitrogen and stored in chloroform. The FAME fractions were treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 ml, 60°C, 1 h) to convert alcohols to their corresponding TMSi (trimethylsilyl) ethers.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC equipped with an HP-1 cross-linked methyl silicone fused silica capillary column (50 m x 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 a 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 30C° min^{-1}, then to 250°C at 2C° min^{-1} and finally to 300°C at 5C° min^{-1}. Peaks were quantified with DAPA Scientific Software. Individual components were identified using GC-MS data and by comparing retention times with those

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obtained for authentic and laboratory standards. GC results are subject to an error of ±5%.

Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a Fisons MD 800 GC-MS fitted with an on-column injector. The GC-MS was operated in scan mode with an ionizing voltage of 70 eV. The GC was fitted with a capillary column similar to that described above.

Determination of double bond configuration in fatty acids and fatty alcohols

Dimethyl disulfide (DMDS) adducts of monounsaturated fatty acids (as methyl esters) and alcohols were formed by treating the total fatty acid and alcohol fractions with DMDS (Dunkelblum *et al.* 1985, Nichols *et al.* 1986). Adducts were then extracted using hexane/chloroform (4:1 v/v) and treated with BSTFA to form TMSi derivatives prior to GC-MS analysis.

Results

Lipid content and composition

The lipid content of whole fish from Elephant Island was 466–585 mg g^{-1} dry weight; fish from Eastern Antarctica contained lonly 394–459 mg g^{-1} dry weight. Myctophids from both areas had oily bones (Table I). Wax esters were the major lipid class present in the skeleton. Lipid from the vertebral centra of *E. antarctica* from both locations had 83.6–87.9% wax esters, whereas that of the neurocranium comprised 67.0–72.8% wax esters. Conversely, the neurocranial lipid had relatively more polar lipids (20.9–29.3%) than the vertebral centra (7.8–13.1%) (Table I).

Despite having oily bones, the skeleton of these fish made up a low percentage of the total body lipid. Neurocranial and vertebral centra lipid of Eastern Antarctic fish together comprised only 3.2±0.8% (as percent total body lipid), whereas visceral lipid was 7.5±1.6% and most body lipid (89.4±0.8%) was stored in the flesh. Likewise, for the Elephant Island fish, 1.6±0.7% lipid (as percent total body lipid), was found in the neurocranium and vertebral central, 10.6±1.3% in the viscera, and 87.8±1.8% of the body lipid was in the flesh.

Flesh samples of *Electrona antarctica* had a very high content of wax esters (WE) (Table I). There were no significant differences in levels of WE between Eastern Antarctica and Elephant Island (ANOVA, $P = 0.59$) or in the levels of wax esters between the tissues analysed ($P = 0.08$). The only substantial amount of triacylglycerols was found in the viscera of Elephant Island fishes (Table I), with some in the skeleton. This exception was due to one sample containing 58% triaclyglycerols, probably reflecting recent feeding of this specimen on prey rich in triacylglycerols. Surprisingly there was no significant difference in levels of triaclyglycerols between Elephant Island and Eastern Antarctica ($P = 0.10$) or in the levels of triaclyglycerols between the tissues analysed ($P = 0.46$). Polar lipids were the second most important lipid class in *E. antarctica*. However, there was no significant difference in levels of polar lipids between *E. antarctica* from Elephant Island and Eastern Antarctica ($P = 0.20$). There were however, significant differences in levels of polar lipids between skull and spine, skull and viscera, skull and flesh, and viscera and flesh ($P = 0.002$). Free fatty acids were low in skeleton and flesh and somewhat higher in viscera in both groups of fish. Free alcohols were low in all fish as were sterols (Table I).

Fatty acid composition

Monounsaturated fatty acids (MUFA) were the most important group of fatty acids in all tissues of all fish analysed (Tables II & III). MUFA in skeletons of both fish groups amounted to 78.9–85.5% (of total fatty acids), flesh MUFA 81.4–85.2%, and visceral MUFA 63.4–73.4%. These MUFA were dominated by oleic acid (18:1(n-9)) and palmitoleic acid (16:1(n-7)). Amounts of these MUFA were similar in the flesh of all fish. The viscera MUFA composition differed substantially from other tissues, particularly in Elephant Island fish. In the viscera of Eastern Antarctica fish, there was 34.8% of 18:1(n-9) and 16.2% of 16:1(n-7). Other

Table I. Percent lipid class of *Electrona antarctica* from Eastern Antarctica and Elephant Island ($n = 3\pm$ sd for all samples except viscera and flesh samples from Elephant Island where $n = 2\pm$ sd).

	Wax esters	Triacylglycerols	Free fatty acids	Alcohols	Sterols	Polar lipids	Lipid as mg g^{-1} dry weight
Eastern Antarctica							
Vertebral centra	83.6±5.8	0.0±0.0	1.7±0.2	0.2±0.0	1.4±0.4	13.1±5.2	234±16
Neurocranium	67.0±10.7	0.0±0.0	1.4±0.5	0.0±0.0	2.3±0.8	29.3±9.5	203±115
Viscera	75.1±5.0	0.7±1.3	6.1±1.8	0.2±0.1	1.1±0.5	16.8±2.7	413±161
Flesh	90.5±1.0	0.0±0.0	1.4±0.2	0.2±0.1	0.3±0.1	7.7±0.9	440±25
Elephant Island							
Vertebral centra	87.9±7.1	1.5±2.6	1.9±0.4	0.7±0.8	0.2±0.2	7.8±3.5	157±34
Neurocranium	72.8±3.3	2.4±2.0	1.7±0.2	2.2±0.4	0.1±0.1	20.9±3.7	326±10
Viscera	50.4±50.8	29.4±40.0	8.7±5.6	0.5±0.7	0.0±0.0	11.0±4.4	446±160
Flesh	86.2±7.7	8.1±11.4	1.7±0.5	0.1±0.1	0.1±0.1	3.9±3.4	577±97

important MUFA in all tissues included 18:1(n-7), 20:1(n-9), 22:1(n-11), and 22:1(n-9) (Tables II & III).

Saturated fatty acids were not a major group. Total saturated fatty acids ranged from 6.8–16.9% (of total fatty acids) in all fishes and had as their principal components 16:0 (3.7–11.6%) and 14:0 (1.4–3.7%) (Tables II & III). Higher levels of saturated fatty acids were found in viscera of Elephant Island fish (16.9% of total) of which 11.6% was 16:0. Polyunsaturated fatty acids (PUFA) were present at similar levels to saturated fatty acids in *E. antarctica* lipid from all tissues. Total PUFA ranged from 7.1–19.4% (of total fatty acids) with eicosapentaenoic acid (EPA, 20:5 n-3, 1.2–6.9%) and decosahexaenoic acid (DHA, 22:6 n-3, 2.9–8.8%) as the principal PUFA.

Fatty alcohol composition

The fatty alcohols were dominated by saturated and monounsaturated alcohols (Tables II & III). Total saturated fatty alcohols ranged from 46.3–54.2% (of total fatty alcohols) (Elephant Island fish) and 37.8–47.8% (Eastern Antarctica fish). The major fatty alcohol was 16:0, with 14:0 as the second most important. There was less 18:0 (2.6–3.7% in all fish). The differences in total monounsaturated fatty alcohols were not significant according to the standard deviation data (Tables II & III). The principal monounsaturated fatty alcohol was 18:1(n-9) whilst second most important was 18:1(n-7). Lesser amounts of the fatty alcohols 16:1(n-7), 20:1(n-9), and 22:1(n-11) were detected (Tables II & III).

Discussion

Total lipid and lipid classes

Electrona antarctica is an oily fish. Specimens from the Elephant Island study area had more lipid than Eastern Antarctica specimens which may indicate more food availability in the Scotia Sea, a rich biological area (Siegel & Loeb 1995). It may also reflect a size (or age) difference, since Elephant Island fish were larger than Eastern Antarctic fish. The level of wax esters in all body parts, excluding viscera, was very high; 90.5% (of total lipid) for Eastern Antarctic and 86.2% for Elephant Island specimens. Triacylglycerols generally accounted for less than 2% of the total lipid in all samples, excluding viscera.

Limited literature data are available on the lipid composition of *E. antarctica*. Reinhardt & Van Vleet (1986) in a study from waters around Croker Passage in the Antarctic Peninsula noted considerably lower levels of wax esters in both whole animals (45–60%) and flesh (62%) and conversely higher levels of triacylglycerols (whole animals, 8–26%; flesh, 18–27%). In the present study, the high levels of wax esters have been confirmed by the TLC-FID analysis in two separate solvent systems. Additional verification was by quantitative GC and GC-MS analysis of component fatty acids and alcohols.

Differences between the Croker Passage fish and specimens from Elephant Island and Eastern Antarctica may reflect variation in diet for fish from the three regions. This would potentially be a significant finding for a circumpolar species,

Table II. Percentage fatty acid and fatty alcohol of *Electrona antarctica* from Elephant Island ($n = 3 \pm$ sd for all samples except viscera and flesh samples where $n = 2 \pm$ sd).

	Vertebral centra		Neurocranium		Viscera		Flesh	
	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol
14:0	2.1±0.2	9.8±0.5	1.4±0.3	7.7±2.5	3.7±0.8	7.3±2.0	2.6±0.7	8.8±0.1
16:0	3.7±0.4	41.1±1.1	4.4±0.7	37.4±8.4	11.6±3.9	34.7±6.0	5.0±0.1	38.5±0.3
18:0	0.9±0.1	2.9±0.2	1.2±0.1	3.1±0.9	1.6±0.4	2.7±0.1	0.9±0.1	2.8±0.4
Sum saturated fatty acids	6.8±0.6	53.9±0.9	7.1±0.9	48.3±11.7	16.9±3.5	44.8±7.2	8.5±0.7	50.1±0.1
16:1(n-7)	18.4±1.5	3.7±0.2	14.1±0.5	3.3±0.9	28.3±26.9	4.0±0.3	20.0±1.9	3.8±0.3
18:1(n-9)	45.3±4.5	25.4±1.0	42.3±2.3	23.7±5.5	14.9±21.1	26.8±7.1	40.2±0.7	23.0±1.1
18:1(n-7)	7.3±0.5	7.1±0.5	7.1±0.6	4.1±3.8	3.7±5.2	6.5±0.6	7.5±1.1	6.4±0.3
20:1(n-9)	3.4±0.2	2.6±0.5	3.6±0.3	2.5±0.9	8.0±8.6	8.5±1.5	5.1±2.4	6.5±4.7
22:1(n-11)	3.0±2.0	1.1±0.4	3.2±2.1	1.0±0.5	2.9±2.7	2.0±0.4	3.4±1.4	1.9±0.7
22:1(n-9)	2.7±1.3	0.9±0.2	2.9±1.3	0.8±0.3	1.9±1.5	1.1±0.1	2.4±1.3	1.0±0.2
24:1(n-9)	1.1±0.3	0.6±0.1	1.9±0.4	0.5±0.2	0.8±0.7	0.5±0.2	0.7±0.1	2.7±3.3
Sum monounsaturated fatty acids	82.2±2.6	41.8±0.8	76.3±1.0	36.2±8.1	61.3±14.8	49.5±6.9	79.8±2.4	45.7±0.0
18:2(n-6)	1.9±0.3	1.3±0.0	1.9±0.1	1.0±0.3	2.6±0.8	0.5±0.7	2.0±0.3	1.2±0.2
20:5(n-3)	1.9±0.5	0.0±0.0	3.4±0.9	0.0±0.0	6.9±6.1	0.0±0.0	2.8±0.7	0.0±0.0
22:6(n-3)	2.9±0.6	0.0±0.0	4.8±0.9	0.0±0.0	8.8±4.7	0.0±0.0	4.3±0.9	0.0±0.0
Sum polyunsaturated fatty acids	7.4±2.1	1.4±0.1	11.5±2.1	1.0±0.3	19.4±10.9	0.5±0.7	9.8±2.7	1.2±0.2
Other	5.5	3.4	5.6	3.2	3.8	5.7	3.0	3.5

Other includes all components present at <2.0%:14:1(n-5), 15:0, i15:0, 16:1(n-7)t, 16:1(n-9), i17:0, br17:1, a17:1, 17:1(n-8), 18:1(n-5), 18:4(n-3), 20:0, 20:1(n-7), 20:1(n-11), 20:2 (n-6), 20:3(n-6), 20:4(n-3), 20:4(n-6), 22:1(n-7), 22:5(n-3), 24:1(n-11).
Monounsaturated components all *cis* geometry.

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but in this instance we propose that variations in methods employed largely account for the observed differences. Open column chromatography separation of wax esters and triacylglycerols, as performed by Reinhardt & Van Vleet (1986), may provide incomplete separation and/or overlapping fractions. We believe this methodological factor probably accounts for the higher levels of triacylglycerols in the Croker Passage *E. antarctica* specimens.

The diet of *E. antarctica* has high levels of wax esters, which consist of 40% amphipods, 30% copepods, and 20% *Thysanoessa macrura* (Hoddell 1996). According to Lee & Hirota (1973), copepods south of 50°S tend to have high lipid, consisting of over 20% wax esters. *Thysanoessa macrura* is also wax ester-rich (Hagen 1988). Amphipods, such as *Themisto gaudichaudii*, are lipid-rich and the principal lipid class is triacylglycerols (Phleger & Nichols, unpublished). The high levels of wax esters in *E. antarctica*, regardless of diet, is indicative of wax ester synthesis, including incorporation of dietary-derived fatty alcohols (Lee & Patton 1989), by this species.

High levels of lipid have been reported in *E. antarctica* from the Weddell Sea/Scotia Sea region of Antarctica (313–503 mg g^{-1} dry weight) (Donnelly *et al.* 1990). These specimens had increased lipid during the summer, compared to the winter months. Childress & Nygaard (1973) reported high lipid levels (as percent dry weight in midwater myctophids off California; 63.3% in *Diaphus theta*, 50.1% in *Triphoterus mexicanus*, and 43.8–44.3% in *Stenobrachius leucopsarus* and *Parvilux ingens* respectively. Nevenzel *et al.* (1969) found high levels of wax esters in three lipid-rich midwater myctophids; 82.2–91.4% wax esters (percent of lipid) in *T. mexicanus*, *Lampanyctus ritteri* and *S. leucopsarus*. Lipid, as mg g^{-1} dry weight in these fishes ranged from 506–564 (Nevenzel *et al.* 1969). More recently, Saito & Murata (1996) found 85.5–87.9% wax esters in the myctophids *Lampanyctus regalis*, *Stenobrachius nannochir* and *S. leucopsarus*. *Benthosema glaciale* and *Gonichys baarnesi* contained 55–87% wax esters (Lee & Patton 1989). Nevertheless, wax esters are not the major lipid class in most myctophid fishes analysed. According to Neighbors (1988), only 15 out of 41 species analysed had wax esters comprising more than 10% of the total lipid. Nine of 41 species had more wax esters than triacylglycerols (Neighbors 1988). The features that result in the synthesis of high levels of wax esters by these nine species, including *E. antarctica*, are not clear. We believe that evolutionary distance, feeding and survival strategies, may be, in combination, key factors.

Buoyancy implications

The functions of wax esters in marine organisms include use as an energy reserve and as a buoyancy agent (Nevenzel 1970). In pelagic copepods, the function of wax esters is primarily as an energy reserve. In zooplankton, wax esters function mostly as an energy reserve, with buoyancy as a secondary function (Lee & Patton 1989). Torres *et al.* (1984), using respiration measurements, calculated that *E. antarctica* could live for 335 days on its stored lipid. However, some midwater fishes, such as the myctophid *Lampanyctus leucopsarus* and stromateoides, have fat invested swimbladders, with wax esters as a major component. In myctophids, lipids take on a major buoyancy function as

Table III. Percentage fatty acid and fatty alcohol of *Electrona antarctica* from Eastern Antarctica ($n = 3 \pm sd$).

	Vertebral centra		Neurocranium		Viscera		Flesh	
	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol	Fatty acid	Fatty alcohol
14:0	1.8±0.2	7.6±0.6	1.6±0.3	8.3±1.1	2.2±0.6	6.4±1.0	1.7±0.2	6.9±0.5
16:0	4.4±0.7	37.2±2.5	5.6±0.8	25.7±17.6	7.2±0.5	36.6±0.6	4.2±0.4	36.4±0.3
18:0	1.2±0.3	2.7±0.3	1.9±0.4	3.7±1.1	2.2±1.1	2.7±0.5	0.7±0.2	2.6±0.3
Sum saturated fatty acids	7.4±0.8	47.8±2.8	9.1±1.1	37.8±15.4	11.5±1.5	46.1±1.1	6.6±0.8	46.3±0.3
16:1(n-7)	18.8±2.7	4.2±0.2	16.5±1.8	4.6±1.4	16.2±0.7	3.7±0.2	20.6±0.2	4.2±0.1
18:1(n-9)	41.9±2.1	24.6±0.7	42.4±0.7	29.0±7.9	34.8±0.7	24.0±1.0	41.0±0.8	25.1±0.8
18:1(n-7)	5.4±0.2	5.6±0.2	5.4±0.3	7.7±1.1	5.7±0.4	6.1±0.9	5.2±0.3	6.1±0.7
20:1(n-9)	6.6±0.7	7.3±0.6	5.6±0.5	8.9±2.3	5.6±0.7	9.3±0.3	6.3±0.6	8.4±0.2
22:1(n-11)	4.5±0.7	2.7±0.3	3.9±0.8	3.4±0.9	3.8±0.9	3.5±0.4	5.1±0.9	3.3±0.2
22:1(n-9)	2.5±0.4	0.9±0.1	2.3±0.4	1.2±0.2	2.2±0.4	1.4±0.1	2.8±0.3	1.1±0.0
Sum monounsaturated fatty acids	80.6±3.0	45.3±0.9	77.3±2.3	55.0±14.9	69.2±2.0	48.0±1.2	82.2±1.2	48.2±0.5
18:2(n-6)	1.5±0.1	1.0±0.1	1.5±0.1	1.3±0.2	1.6±0.1	1.1±0.1	2.2±0.4	1.4±0.1
20:5(n-3)	1.7±0.6	0.0±0.0	2.1±0.3	0.0±0.0	4.6±1.3	0.0±0.0	1.2±0.3	0.0±0.0
22:6(n-3)	3.1±1.3	0.0±0.0	4.2±1.2	0.0±0.0	7.6±1.3	0.0±0.0	4.2±0.2	0.0±0.0
Sum polyunsaturated fatty acids	6.8±2.2	1.0±0.1	8.3±1.5	1.3±0.2	14.3±2.6	1.1±0.1	7.8±0.5	1.4±0.1
Other	6.6	6.2	6.3	6.0	6.4	5.3	4.9	4.6

Other includes all components present at <2.0%: 14:1(n-5), 15:0, i15:0, 16:1(n-7)t, 16:1(n-9), i17:0, br17:1, a17:1, 17:1(n-8), 18:1(n-5), 18:4(n-3), 20:0, 20:1(n-7), 20:1(n-11), 20:2 (n-6), 20:3(n-6), 20:4(n-3), 20:4(n-6), 22:1(n-7), 22:5(n-3), 24:1(n-11).
Monounsaturated components all cis geometry.

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the swimbladder regresses with age and becomes invested with fat (Butler & Pearcy 1972). The wax ester-rich swimbladder of the orange roughy, *Hoplostethus atlanticus*, is fat invested (Phleger & Grigor 1990) as in the Latimeridae and Myctophidae, which also contain large amounts of wax esters (Nevenzel *et al.* 1966, 1969). Furthermore, wax esters are extracellular in fish like orange roughy (Phleger & Grigor 1990) and extracellular lipid may have buoyancy as its sole function because non-digestive lipases are intracellular (Phleger 1991).

Oily bones

Electrona antarctica has oily bones and the bone lipid is primarily wax esters, which is similar to the body lipid composition of the fish. It is unusual to find wax ester perfused bones; most fish with oily bones have triacylglycerols as the major lipid class in the skeleton (Phleger & Wambeke 1995). Nevertheless, the bones of orange roughy, *Hoplostethus atlanticus*, are filled with wax (Grigor *et al.* 1990, Phleger & Grigor 1990), and the bones of the coelacanth, *Latimeria chalumnae* and the castor-oil fish, *Ruvettus pretiosus*, are possibly also wax-filled (Nevenzel *et al.* 1966, 1965). Although the blackbelly rosefish, *Heliocolenus dactylopterus lahillei*, has 21.1% wax esters (as percent of the lipid) in the muscle, triacylglycerols are the major lipid (84.4–87.8%) in the skeleton (Mendez *et al.* 1993). These authors suggest that the lipid-rich poorly calcified bones exert buoyancy control, and that the bone triacylglycerols play a role in energy reserve.

Fatty acids and alcohols

The high levels of MUFA observed in *E. antarctica* (63.4–85.5%, of total fatty acids) are similar to levels observed in the myctophids *Stenobrachius leucopsarus*, *S. nannochir*, and *Lampanyctus regalis* by Saito & Murata (1996). MUFA accounted for over 90% of the fatty acids in the orange roughy, another wax ester-rich fish (Bakes *et al.* 1995, Elliott *et al.* 1990). Nevenzel *et al.* (1969) also found high MUFA levels in myctophid wax esters; fatty acid profiles were dominated by octadecenoic acid (18:1) (71.5–76.4%). The fatty acids of wax esters are typically low in PUFA, (7.1–19.4%) which agrees with studies cited above. Our fatty alcohol data are also similar to results of Nevenzel *et al.* (1969) and Saito & Murata (1996) with 16:0 and 14:0 being the dominant saturated fatty alcohols and 18:1(n-9) and 18:1(n-7) being the major monounsaturated fatty alcohols.

The ratio of 22:1 to 20:1 fatty alcohols can provide further insight on diet. A comparison of this ratio for orange roughy showed values of >2 for North Atlantic fish and <1 for Australian fish (Bakes *et al.* 1995). For *E. antarctica* from both the Elephant island and Eastern Antarctica regions, the 22:1/20:1 ratio was <1. The high ratio for the North Atlantic orange roughy was proposed as resulting from a diet comprised of calanoid copepods rich in wax esters and 22:1 alcohol. As for the Australian orange roughy, the low ratio of 22:1/20:1 alcohols observed for *E. antarctica* is consistent with a diet of amphipods, copepods and other prey items low in 22:1 alcohols.

The relative level of the nutritionally important ω3 fatty acids EPA and DHA in the flesh of *E. antarctica* was 5.4% and 7.1% for the Eastern Antarctic and Elephant Island fishes respectively (Tables II & III). These values are considerably lower than levels determined for many marine fishes (e.g., Bremner *et al.* 1989, Nichols *et al.* 1994, Nichols *et al.* 1996 and unpublished data).

On an absolute basis, *E. antarctica* flesh also contained low levels of polyunsaturated fatty acids. In Eastern Antarctica, *E. antarctica* flesh had 59±2 μg DHA g^{-1} and 17±3 μg EPA g^{-1} wet weight. Lower amounts of these fatty acids were found in fish from Elephant Island which had 18±6 μg DHA g^{-1} and 12±3 μg EPA g^{-1}. These levels cannot be compared to other Antarctic fish because in the few studies done, absolute levels are not reported. Absolute levels of both EPA and DHA in *E. antarctica*, however, are low compared to those reported for arctic and temperate fish (e.g. Ackman 1992, Pickston *et al.* 1982, Sigurgisladottir & Palmadottir 1993).

The PMS (polyunsaturated:monounsaturated:saturated) ratio of 1.0:9.6:1.0 (Elephant Island) and 1.2:12.9:1.0 (Eastern Antarctica) is more favorable than most other flesh foods (English & Lewis 1991, Woodward *et al.* 1995). Indices of atherogenicity and thrombogenicity can be calculated for foods based on their fatty acid composition (Ulbricht & Southgate 1991). For *E. antarctica* flesh, atherogenicity and thrombogenicity indices of 0.12–0.17 and 0.1–0.13 respectively were determined. Due to the high levels of monounsaturated fatty acids in *E. antarctica* flesh (81–84%, Tables II & III), these ratios are extremely favourable relative to beef, chicken and polyunsaturated margarines and also compare very favorably with fish species containing elevated levels of ω3 fatty acids.

As the oil of *E. antarctica* is rich in wax esters, which may not be fully hydrolyzed by humans and other mammals (Place 1992), the bio-availability of wax fatty acids may be less than 100%. Wax ester oils theoretically may cause mild steatorrhea, with the wax esters excreted unchanged. Based on feeding trials of orange roughy to pigs, it was considered that normal consumption by humans is unlikely to cause any serious health problems (James *et al.* 1986).

Food chain and fisheries implications

Myctophids are very important in the diet of Antarctic seabirds and mammals. According to Hopkins *et al.* (1993), *E. antarctica* was the major food item for seven of nine dominant seabird species examined. Midwater fishes, especially *E. antarctica*, are more important than krill as food to flying seabirds feeding in open water near the ice edge. Pelagic fishes, mostly myctophids, were consumed at

the rate of 1.7 x 10^6 kg^{-1} per day by penguins (74% king penguins, 21% macaroni penguins) breeding at the Prince Edward Islands (Adams *et al.* 1993). Adams & Brown (1989) reported that King penguins from the sub-Antarctic Marion Island also consumed primarily myctophid fish including *Electrona carlsbergii* (also *Kreffichthys anderssoni*, *Protomyctophum tenisoni*, and *P. normani*) which occurs north of the Antarctic Convergence (40°–68°S, in the South Pacific; Wisner 1974). Both seabird and seal predators consumed about 250 000 tons of myctophids annually in the South Georgian region (CCAMLR 1991). *Electrona antarctica* has a circumglobal distribution, and is usually found south of the Antarctic convergence (Wisner 1974). The diet of Antarctic fur seals in the South Orkney Islands consisted of 93.4% myctophid fishes, mostly *E. antarctica* and *Gymnoscopelus nicholsi* (Daneri & Coria 1993).

According to Sabourenkov (1991) the biomass of mesopelagic myctophids south of 40°S is 70–396 x 10^6 tons. Commercial fishing of myctophids started recently in the Atlantic section, and there are probably commercially exploitable stocks of lanternfishes in the Pacific and Indian section of the Southern Ocean (Cherel *et al.* 1993). In 1991, 78 488 tons of myctophids (mainly *E. carlsbergii*) were caught in the South Georgian subarea which made up 95% of the total finfish caught in this region (CCAMLR 1991). Large stocks of lanternfishes have been reported from hydroacoustic surveys in the Benguela Current off Africa (Cruickshank 1983) and feasibility studies of a possible Iranian fishery for myctophids have been conducted (Shotton 1996).

Wax ester-rich oils have important commercial uses. Wax esters from the orange roughy have been exported to Japan for use as lubricants in the steel industry (Nichols *et al.* 1994) and have also been incorporated into a variety of degreasing and cleaning products for the local Australia and exports markets. Wax ester oil has high stability at elevated temperatures in contrast to the more common triacylglycerol-rich fish oils. Marine wax ester oils have also been used in cosmetics as a substitute for jojoba oil which has been used as a replacement for sperm whale oil, and sold in Japan for Aust $25 per kg (Nichols *et al.* 1994). Since the oil from *E. antarctica* is rich in monounsaturated components, like orange roughy oil, it should have industrial uses and therefore similar commercial potential.

In summary, *E. antarctica* is an oily fish particularly rich in wax esters. Lipid compositional data for *E. antarctica* has provided comparative information on aspects of the biochemical dynamics and trophodynamics of this species. In addition, the data also may be pertinent for evaluation of commercial opportunities for the direct harvest, or as a secondary-catch, of *E. antarctica* from Southern Ocean waters. As such, these data also may be directed towards ensuring maximum return is gained at whatever catch levels are considered to be sustainable for *E. antarctica*.

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