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ORANGE ROUGHY AND OTHER MARINE OILS: CHARACTERIZATION AND COMMERCIAL APPLICATIONS

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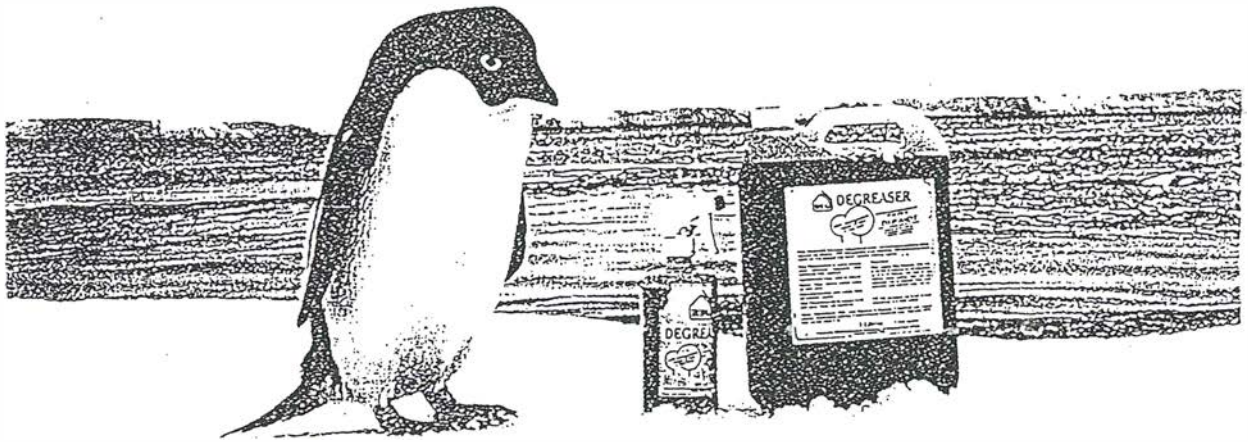

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(Manuscripts are attached to the report; associated manuscripts are available on request, and reports are held by the CSIRO Marine Laboratories)

Orange Roughy and other Marine Oils:

Characterization and Commercial Applications

1.0 Summary

Research undertaken by the CSIRO Marine Products project drew together the combined expertise of CSIRO and industry with the objective of increasing the value of the bycatch and by-products of Australia's south-eastern fisheries, in particular the larger, and economically important catches of orange roughy, oreos and other species including deep-sea shark.

Oil products which have been developed or have the potential for development in Australia include: (i) oils rich in wax esters from orange roughy, oreos and other species, (ii) squalene and other components from the livers of deep-sea sharks and (iii) oils enriched in omega-3 polyunsaturated fatty acids (PUFA). Commercial products have been developed overseas from these three types of oils. The lipid composition of fish can depend, however, on seasonal changes in reproductive status and feeding habits, changes in available food, regional differences in the basic foods and nutrients, and other factors. It is due to these possible variations that there was a need to characterize the oil derived from Australian fish.

With the aid of FRDC funding (grant 91/77), the Marine Products project of the CSIRO Division of Oceanography has developed a considerable intellectual resource on Australian marine oils. State of the art laboratory facilities and expertise have been assembled for the chemical characterization of oils. This research has assisted a number of companies that have established facilities for the production of oil and high grade meal from the south-east Australian fisheries.

The research has enhanced post harvest production of Australia's fisheries and assisted in converting what was waste material into value-added by-products. Such products include squalene from shark liver oil, omega-3 PUFA from conventional fish oils, and speciality products from wax ester rich oils. The economic yields of Australia's fisheries can be increased and environmental concerns minimized with utilization of waste material.

Research undertaken with FRDC grant 91/77 has provided major impetus to the relatively young Australian marine oils industry, with an emphasis on first, orange roughy oil, then on shark liver oil. Further research is still required on other species, as is more refined work on species. The research effort is needed to enable the small businesses involved in this industry to continue to be viable. For Australian industry to achieve an economically sustainable level of value-added products, there is a need to strengthen our knowledge of the oil composition of new commercial and bycatch species and also on species (e.g. various tuna and mackerel) which offer potential for both increased catch and utilization, including oil production and value-adding.

The principal benefactors of the research to date have been the individual companies attempting to exploit the marine oil resources associated with the by-products and bycatch

from the Australian fishing industry. The fishing industry itself will similarly develop as new markets are found for by-products and bycatch. The community at large will benefit through the more efficient usage of by-products and bycatch, and reduction in waste, as well as through the possibility of cheaper local sources of refined marine oil products. Finally the Australian economy will benefit through the production of value-added export products, which can also potentially replace materials imported into the country.

Results have been communicated in the form of: (i) specific biochemical data on individual species, oils, oil fractions and related samples, and (ii) processing technology or expertise developed. Various means were used to communicate results to the fishing and related industries, including:

- Reports on specific analyses carried out for industry.
- Direct personal communication to industry.
- Scientific presentations at conferences, workshops and seminars.
- Manuscripts in *Australian Fisheries* and in other national and international journals.

A list of publications, reports and conference presentations on marine oils during the period of project FRDC 91/77 is provided in the Appendix.

During this FRDC funded project, strong ties have been established with several companies involved in fish processing or value-added fish by-products. These include for example:

- BEKU Environmental Products Ltd who we have assisted with analytical data on their marine-oil based degreasers and cleaning products.
- Trident Seafoods who we have provided new compositional data on the oils and flesh from potential commercial southern ocean species.

Tas Crays who have produced a soil additive from orange roughy waste and we have assisted with various analytical data; Squalus.
BEKU and Scales who we worked with on the development of shark liver oil and squalene products. The latter three companies were the primary contacts for shark liver oil products, however, results also have been made more widely available. As the fishing industry became more aware of our research, we responded to many requests for information from fishermen, processors in Tasmania and mainland states and related industries.

At the time of preparation of this report, discussions had been held with several companies on the potential to manufacture a value-added omega-3 PUFA product in Australia. Industry cannot afford to undertake the research on its own, nor does it have the technology or expertise to undertake the required research. Negotiations have been of a preliminary nature only and no decision or agreement has been made on extending the results to industry.

Risks associated with the development of marine oil products are predominately beyond our control in that expansion of the Australian marine oil industry will be dependent upon continuity of fisheries, international market factors and other economic considerations. The results of this FRDC project will, however, ensure that Australian industry has the best possible scientific and technical information available to help overcome such risks.

Achievements have included:

- a large number of Australian companies have been provided with advice, assistance and analyses of oil and related samples.
- surveys of the oil composition of orange roughy, oreo dory and other species.

- increased information of the oil composition of southern sharks.
- expansion of the data base on the composition of marine oils derived from Australian fish.
- development of a process to separate squalene from shark liver oil (Australian Provisional Patent PL6103/92).
- the transfer of this and other technologies, related advice and other information to industry.
- the development (and marketing) by industry of new value-added marine oil products deriving from Australian species.
- development of laboratory methods for enrichment of diacylglyceryl ethers from shark liver oil.
- study of the omega-3 PUFA composition of Australian species.

2.0 Rationale and background

The discovery of the first orange roughy spawning aggregation in Australian waters in 1989 resulted in the largest commercial catches taken to date for this species. Combined catches from northeastern and southern Tasmania in 1989-90 exceeded 34000 tonnes; just under half of the catch came from the spawning aggregation off St. Helens. A further 16000 tonnes was taken from the spawning aggregation in winter 1990. A spawning aggregation, from which 800 tonnes was caught, was located in the Great Australian Bight in July 1990. Of the orange roughy catch, approximately 30% is processed into fillets. The remainder of the fish was at that time largely discarded as waste.

Until this study was commenced, only limited work had been undertaken within Australia on further processing of orange roughy waste. This was due to a lack of information on possible uses and a general lack of high quality biochemical data on orange roughy and the waste obtained from processing. A major aim of the FRDC-funded study (91/77) was to provide such data to assist industry in the further development and utilization of the orange roughy waste resource. The intention was that the knowledge developed would be readily transferred to the utilisation of by-products and by-catches from other fisheries.

Orange roughy waste contains two components of value: oil and pigment. First, orange roughy contain copious quantities of oil; approximately 18% of the whole fish consists of oil. About 80% of this oil is located in waste products - the head, swim bladder, frame and skin. Over 2,500 tonnes of crude oil were discarded with the waste material of the 1989 catch alone. In 1989, this oil was worth $2.5 M based on an estimate of $1 per kg; the refined oil is worth more.

The second component of value in the waste is the pigment. A primary concern for the salmon aquaculture industry worldwide is the production of fish with the correct redness of flesh. Wild salmon have flesh with a characteristic redness, however farmed salmon require the addition of pigment (carotenoids) to the feed. As a local source of the red pigment is not available, commercially produced synthetic pigment is added to the food used in the Australian aquaculture industry. The high cost and low quality of the pigment currently used indicates that alternate sources of the carotenoid pigments should be sought. The orange coloration of orange roughy may provide a source of carotenoids for the feed for salmon and other farmed animals which require the pigment. One aim of this study was to characterise the pigment and develop methods for its isolation.

Orange roughy waste can be used as a plant growth medium. Production of a soil additive following composting of orange roughy waste with eucalypt waste was commenced by Tas Crays, Margate. New Zealand industry had previously composted orange roughy with pine waste. The orange roughy waste is mixed with plant material and applied to soil in horticultural practices. Such processing is more desirable than dumping the waste, causing environmental problems as happened in 1989 in Tasmania. Analysis of waste oil obtained from this process was to be conducted, to assist in determining uses for this material.

Even if the 1989 and 1990 catch levels of orange roughy are not sustained, all indications are that a major deepwater fishery will continue in Australia. Hence there will a resource of material for possible oil, pigment, meal and fertilizer production. By enhancing post

production of orange roughy and testing the likelihood that what is now waste material can be converted into value-added by-products, the proposed study will maximize economic yields of the fishery at whatever level of harvest is achieved.

Deep sea sharks are an under-utilized by-catch of the orange roughy fishery. When orange roughy are not abundant, up to 50% of the catch may be sharks. The livers of these sharks are large (about 20% of the total shark's weight), and they contain considerable quantities of oil which is enriched in the C_{30} unsaturated isoprenoid hydrocarbon squalene. Squalene can be hydrogenated to squalane which is used in the pharmaceutical and cosmetic industries, particularly as a lubricant and cosmetic base. When the study was commenced, small quantities of shark liver oil were being sold unprocessed into the Japanese market for about $5/kg. There is scope to increase the amounts exported and to further refine the oil into value-added products. Shark liver oils also contain diacylglyceryl ethers (DAGE). The liver of *Centroscymnus squamosus* contained 79% DAGE, but contents around 20-40% are more common. These compounds are similar to triacylglycerols, but one of the ester bonds is replaced by an ether bond which makes the compounds more stable. Australian industry presently imports these compounds from overseas, so there is potential for import replacement and for the development of an export market.

Full exploitation of this resource will depend on a better understanding of which species contain marketable quantities of squalene and diacylglyceryl ethers. Previous studies were restricted to 8 species commonly caught in waters to the west and east of Tasmania. In 3 cases, only a single specimen was studied. This data base needs to be expanded to include a statistically useful number of samples and extended to other species, including those caught in other Australian waters.

Orange roughy oil and shark liver oils, together with oils derived from other deepwater species, have specialist applications because of their unusual compositions. Most marine oils, however, consist of triacylglycerols which are rich in polyunsaturated fatty acids. The markets for marine oils continue to expand, but too little is known about the oil content and composition of most Australian fish. For example, oreos are now caught commercially in the same areas as orange roughy, however limited information is available on the oil composition of these fish. We have conducted studies on species marketed in Tasmania, but these have been restricted to fish for human consumption rather than analyses of trash fish or of waste products. We believe that it is this latter area that offers scope for future commercial development.

Polyunsaturated fatty acids have been shown to reduce the incidence of coronary heart disease and stroke in humans, which has considerably enhanced the image of fish as a healthy food amongst consumers. Capsules of fish oils containing high levels of essential polyunsaturated fatty acids are marketed overseas. The demand for fish oils by the mariculture industry will also increase. The economics of fish farming rely on the supply of inexpensive feeds of a suitable quantity and quality. Tasmania has fortunately had large catches of jack mackerel, but when this fishery failed in 1989 oils had to be imported at considerable cost. At that time, data were not available on possible local replacements. By building the data base now on marine oil compositions, we will be much better placed to identify alternative feedstocks and to take advantage of new market opportunities.

3.0 **Objectives** *(as set out in original proposal)*

(1) To identify the red pigment material present in orange roughy, determine the yield of the pigment, and examine means to separate the pigment from orange roughy oil.

(2) To characterise the chemical composition of orange roughy oil from Australian caught fish and compare the oil composition with that obtained for New Zealand and other regions. This will include characterisation of oil from various geographic regions and spawning and non-spawning fish, oil from various organs, and fish sizes. Oil obtained from different processes will be analysed.

(3) To use the methods developed for (2) to assist in monitoring breakdown of orange roughy oil during composting of orange roughy waste.

(4) Determination of the composition of other marine oils to determine their suitability as sources for polyunsaturated fatty acids and other speciality chemicals.

(5) Characterisation of the composition of shark liver oil collected from different fishing areas, and an examination of methods for further refining squalene and diacylglyceryl ethers.

Personnel involved in project

Peter Nichols	PhD	Project leader	20%	
John Volkman	PhD		5%	
Nick Elliott	PhD			5%
David Nichols	BSc (Hons)	1992 only	100%	
Michael Bakes	BSc (Hons)	1993 only	100%	

4.0 Results and discussion

Value-added marine oil products can be manufactured from both the waste generated during sea-food processing and the bycatch of established and new fisheries. Such products have uses in the aquaculture, pharmaceutical, food and other industries. To date, most of the research on marine oils has focused on processing by-products of commercial species with little emphasis on the possible utilization of the bycatch from major fisheries that is usually dumped by industry. There is increasing government and public concern for minimizing waste generated by industry, including from aquaculture and seafood processing. This provides a stimulus for the production of further value-added marine oil products from underexploited species and from waste generated by the Australian fishing industry.

Specialised analytical techniques have been developed for the characterization of marine oils. Details on methods commonly used during the project are provided in appended scientific manuscripts and will not be repeated here. The results described in this report summarize more detailed data sets presented in the attached manuscripts and other data available in many of reports prepared for industry (listed in Appendix).

4.1 *Orange roughy oil and pigments (objectives 1, 2 & 3)*

Our initial work showed for the first time that the oil present in orange roughy caught in Australian, New Zealand and British waters (the latter two data sets were from the literature) had a similar component distribution. The oil is rich in monounsaturated constituents and, compared with most fish oils, it contains very low levels of polyunsaturated fatty acids (PUFA). The major fatty acids in the commercially-produced oil in decreasing order of abundance are: 18:1(n-9)c, 20:1(n-9)c, 16:1(n-7)c, 18:1(n-7)c and 22:1(n-11)c. The major alcohols are: 20:1(n-9)c, 16:0, 22:1(n-11)c and 18:1(n-9)c (Table 1). These results were good news for Australian companies previously importing oil from New Zealand, in that there was a local source available. These results have been passed on to local processors. Our early work also highlighted variations in the minor components of the oil with fish size and location; the latter may prove useful in defining discrete "aggregations" of orange roughy.

A second set of analyses were carried out on the muscle and swim bladder of *Hoplostethus atlanticus* from both Australian and North Atlantic waters. Orange roughy collected from Australian waters were distinguished from their North Atlantic counterparts based on lipid and fatty alcohol profiles. Total lipid in flesh from North Atlantic fish was much higher than in Australian fish (27% versus 3% wet weight, respectively); this may be a sex factor, which is currently under investigation. In comparison, swim bladders show similar lipid content (Table 2). The ratio of 22:1 to 20:1 fatty alcohols in orange roughy from the two regions was 0.5 (Australian) and 1.4 (North Atlantic). The changes in the ratio of 22:1 alcohol to 20:1 alcohol (Table 3) are most likely attributed to differences in diet; presumably calanoid (high wax ester, high 22:1) in North Atlantic fish and smaller fish and squid (lower wax ester, lower 22:1) in Australian fish. These data are being prepared for publication in *Australian Fisheries* and the international literature.

Table 1. Comparison of the composition of the major fatty acids and fatty alcohols of commercially produced oil and oil from the swim bladder of orange roughy caught off Australia, New Zealand and Britain. The data are expressed as a percentage of total fatty acids or fatty alcohols.

Constituent#	Commercial oil		Australia1		New Zealand2		Britain3	
	alcohol	acid	alcohol	acid	alcohol	acid	alcohol	acid
14:0	2.0	0.1	3.8	1.8	1.7	1.7	2.3	0.8
16:0	21.4	1.7	30.8	2.3	24.2	2	24	1.6
16:1(n-7c*)	1.0	10.3	0.6	12.3	3.6	13	2.1	10.9
18:0	6.3	0.5	7.6	0.9	7.3	0.8	4.6	0.1
18:1(n-9c*)	13.8	47.8	15.7	46.2	17.4	54	17	50.8
20:1(n-9c*)	26.0	14.3	23.5	17	26.7	17.1	22	18.3
22:1(n-11c*)	12.6	4.4	8.7	4.7	16	7.9	28	14.6
Others	16.9	20.9	9.3	14.8	3.1	3.5	-	2.9

1. This study (mean of 5 fish, all 37 cm, 1.75 to 2.1 kg); 2. Ref. Grigor et al., 1983 (mean of 6 fish, 0.5 to 1.9 kg); 3. Ref. Sargent et al., 1983 (20 cm, 0.3 kg).
- refer to footnote; * - cis geometry, this study only.

Table 2. Wax ester content of Orange roughy samples.

Sample	Flesh	Swim Bladder
North Atlantic	mg/g (wet)	mg/g (wet)
94	390	759
95	272	721
96	121	1427
97	314	779
98	352	772
99	345	869
100	117	945
Mean	273	896
Tasmanian		
1184	40	815
1185	48	822
1187	34	
1191	11	
Mean	33	819

Table 3. Comparison of the major fatty acids and fatty alcohols in wax esters from the swim bladder and muscle of orange roughy caught in North Atlantic waters and in wâters off Australia. (The data are means for number of fish shown and are expressed as percentage of total fatty acids or alcohols ; -not detected).

Constituent	North Atlantic		Australia	
	swim bladder	muscle	swim bladder	muscle
Number of fish	7	7	2	4
Fatty acids (%)				
14:0	1.1	1.6	1.8	nd
16:0	1.2	1.6	1.9	2.8
16:1(n-7)	6.3	6.4	10.1	8.4
18:1(n-9)	25.4	28.4	49.5	56.2
18:1(n-7)	3.9	4.2	4.3	5.7
20:1(n-9)	29.1	23	21.2	17.2
22:1(n-11)	29.7	31	8.2	9.1
22:1(n-9)	2.1	2.5	1.7	-
20:5(n-3) (EPA)	0.6	0.7	1.3	0.7
22:6(n-3) (DHA)	0.5	0.5	0.5	-
Fatty alcohols (%)				
14:0	1	0.7	1.9	1.4
16:0	11.6	9	23.6	24.2
18:0	3.7	4	7.4	9
18:1(n-9)	5.3	4	13.4	12.9
20:1(n-9)	23.2	20.3	29.6	26.5
22:1(n-11)	47.2	52.7	15.6	17.3
22:1(n-9)	4.3	5.1	4.3	4.7
24:1	3.6	4.2	4.3	4.1

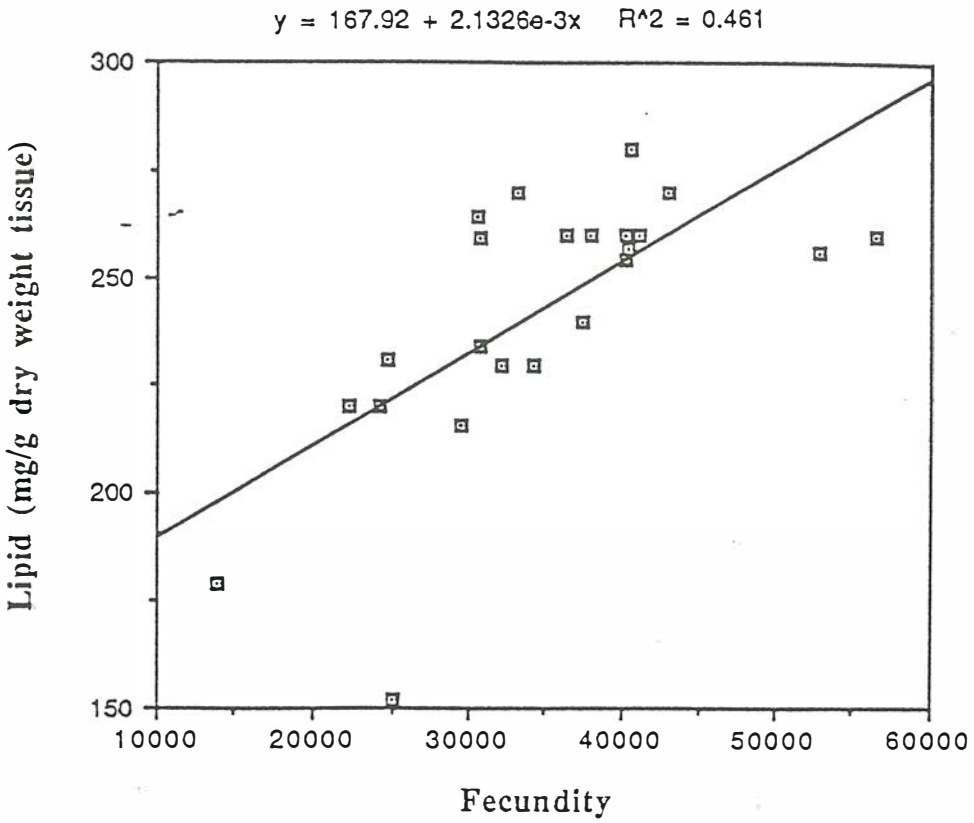

Figure 1. Relationship between lipid (mg/g dry weight tissue) in the ovary of Orange Roughy (Hoplostethus atlanticus) and fecundity (total eggs per ovary).

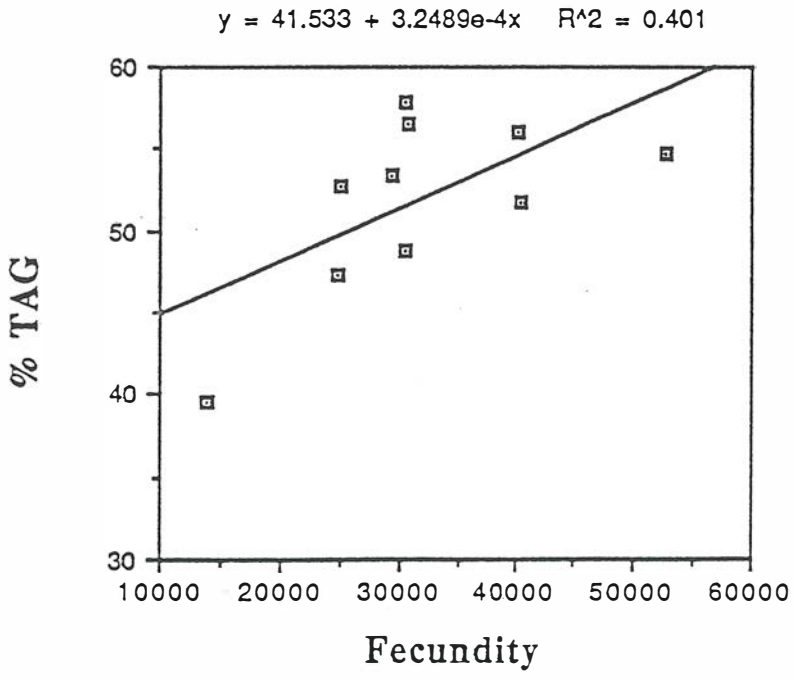

Figure 2. Relationship between triacylglycerol (percentage of total lipid) in the ovary of Orange Roughy (Hoplostethus atlanticus) and fecundity.

In a separate study, the relationship between orange roughy fecundity and the lipid content and composition of muscle, liver and ovary was also investigated. Muscle lipid content ranged from 9.5% to 61% dry weight with an average of 40% (n=9). Liver lipid content averaged 41% of the total lipid which ranged from 16% to 61% (n=9). There was no significant correlation between the muscle and liver lipid levels with fecundity. Ovary lipid content significantly increased with increasing fecundity ($p>0.01$) from 15% to 27% dry weight (Figure 1), with an average value of 23% (n=22). Wax ester was the major lipid class of muscle tissue comprising up to 99% of the total lipid. Triacylglycerol however was the major constituent of the liver which ranged from 38 to 79% of the total lipid with wax esters ranging from 7 to 20%. Triacylglycerol, the major lipid class in the ovary, increased significantly ($p>0.05$) with increasing fecundity (Figure 2) ranging from 40 to 58%. Conversely, polar lipid decreased with increasing fecundity, with levels ranging from 41 to 27%. Wax esters were a minor component of the ovary tissue with average levels of 7%. The saturated and monounsaturated fatty acids in the ovary averaged 33% and 54% of the total fatty acids respectively. The long chain polyunsaturated fatty acids; eicosapentaenoic (EPA) and docosahexaenoic (DHA) averaged 2% and 5% respectively of the total fatty acids.

Anecdotal reports by fisherman provided evidence of the solvating properties of orange roughy oil. Wet weather gear was rotted, and loose paint and greases were removed from metal surfaces by the oil. BEKU Environmental Products Ltd., working in association with CSIRO, has developed biodegradable cleaning products using marine oils which are now being marketed. The products were successfully trialled at the Australian Antarctic bases Mawson and Davis during the summer of 1991/92 (Figure 3). The degreaser and hand-cleaner products compete with currently used petrochemical or organochlorine-based products. The latter are considered to be less biodegradable and more toxic than the marine oil-based products.

The composition of orange roughy oil makes it a possible substitute for jojoba oil which has been used as a replacement for sperm whale oil. These oils are utilised by the cosmetic and pharmaceutical industries after being refined, decolorized and deodorized. At present, the oil is refined in Japan and sells for Aust $25 per kg. This is an area for future development and there is scope to produce the value-added high-quality oil in Australia. For example, overseas haircare products now exist based on Australian orange roughy oil; there is no obvious reason why such products cannot be manufactured in Australia.

The waste oil that drains off during the composting of orange roughy waste with eucalypt waste has also been analysed (Table 4). The high wax ester content of the oil is maintained during composting as wax esters are considerably more stable than conventional (triacylglycerol-containing) fish oils. After appropriate treatment, this oil may also be suitable for use by industry.

The pigments from orange roughy oil were analysed by several procedures. The main component was found to be an astaxanthin ester. Attempts were made to purify the pigment from the orange roughy wax ester rich oil under standard laboratory conditions. These efforts produced product at extremely low recovery; this is due largely to the similar physical properties of the carotenoid pigment and the wax exter present as the dominant constituent of the oil. At the present time the unrefined pigment could be of use to the mariculture industry as natural sources are sought for the astaxanthin ester. Further research and development will be required to produce a purified pigment product.

Table 4. Lipid class composition of commercially produced orange roughy and oreo oils and waste oil produced during composting of orange roughy waste with eucalypt waste.

Lipid Class	Percentage composition		
	Orange roughy oil	Waste orange roughy oil	Oreo oil
Wax ester	97.1	92.8	90.9
Triacylglycerol	1.9	-	9.1
Free fatty acid	Not detected	4.1	-
Fatty alcohol	Not detected	2	-
Sterols	-	-	tr
Polar lipid	1	1.1	tr

tr: trace, <0.5%

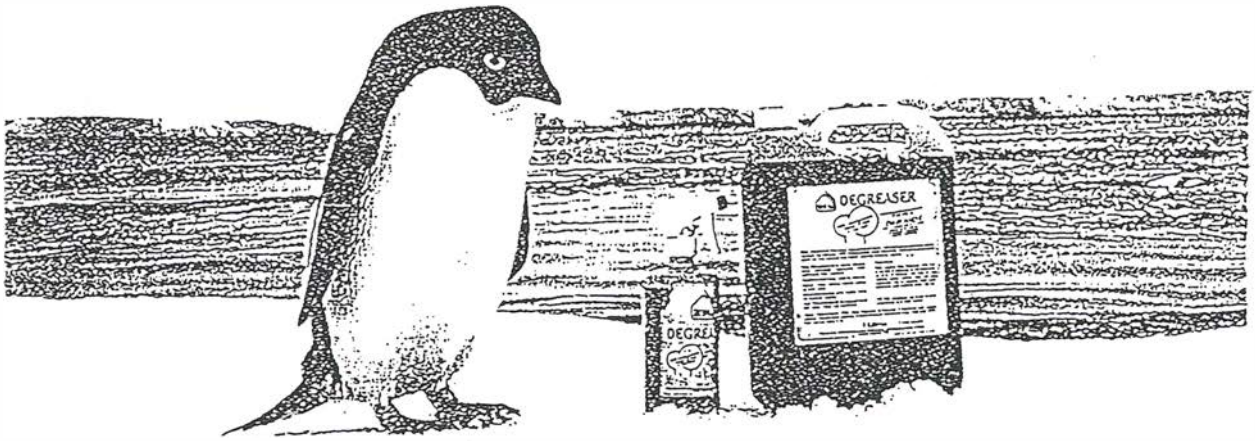

Figure 3. Antarctic field trials of orange roughy oil based degreaser. BEKU Environmental Products Ltd., working in association with CSIRO, has developed biodegradable cleaning products using marine oils. The products were used at the Australian Antarctic bases Mawson and Davis during the summer of 1991/92. The degreaser and hand-cleaner products compete with currently used petrochemical or organochlorine-based products. The latter are considered to be less biodegradable and more toxic than the marine oil-based products.

Even with the reduction in the catch levels of orange roughy, all indications are that a major deepwater fishery is likely to continue in Australia. Hence there will continue to be a resource of material for possible oil, pigment, meal and fertilizer production.

4.2 *Oreo oil (objective 4)*

The lipid, fatty acid and fatty alcohol composition was determined for muscle samples from seven species of deep-sea oreo collected from Australian waters; namely *Neocyttus rhomboidalis*, *Neocyttus* sp., *Allocyttus verrucosus*, *Allocyttus niger*, *Pseudocyttus maculatus*, and *Oreosoma atlanticum*. *Neocyttus helgae*, landed in North Atlantic waters, was also analysed. In the oreos, the mean lipid content ranged from 0.5 to 3% of wet weight; a mixed lipid composition including wax ester, triacylglycerol, sterol and polar lipid was observed (Table 5).

The large degree of within species and between species variation in lipid content and composition is probably due to environmental and biological factors. All oreos contained elevated levels of the essential polyunsaturated fatty acids, EPA and DHA (Table 6); this will be a useful marketing feature for the oreos. There were no clear distinguishing features between species, however, the ratio of the monounsaturated fatty alcohols 22:1 to 20:1 did distinguish samples collected from the two geographical regions (greater than unity for N. Atlantic species and less than unity for Australian species; Table 7), substantiating previous dietary relationships related to the origin of these alcohols. The ratio of 22:1 alcohol to 20:1 alcohol was not species specific and is again most likely attributed to differences in diet; presumably calanoid (high wax ester, high 22:1) in North Atlantic fish and smaller fish and squid (lower wax ester, lower 22:1) in Australian fish, as observed for orange roughy.

The composition of oils produced commercially from oreos was also determined. The oil from oreos was found to be rich in wax ester (Table 4); this oil, like orange roughy oil, is rich in monounsaturated components (Figure 4). The fatty acid and alcohol distribution differs slightly from that found for orange roughy, however, it will be possible to substitute or at least blend the oil with that produced from orange roughy.

4.3 *Shark liver oil (objective 5)*

Previous analyses of shark liver oil had been conducted independently by the CSIRO Marine Laboratories. In association with Australian industry, a further survey of important commercial deep sea shark species was conducted during this FRDC study.

The liver oils from the deep-sea sharks *Somniosus pacificus*, *Centroscymnus plunketi*, *Centroscymnus crepidater*, *Etmopterus granulosus*, *Deania calcea* and *Centrophorus scalpratus* were analysed to describe their lipid, fatty acid and squalene compositions. The major lipids in all species of shark were diacylglyceryl ethers and triacylglycerols and the major hydrocarbon was squalene. Only trace levels of polar lipids were detected. Monounsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{20:1}$, $C_{22:1}$ and $C_{24:1}$) comprised 62-84% of the fatty acids. Saturated fatty acids contributed 11-26% of the total fatty acids while polyunsaturated fatty acids were relatively minor components (1-13%). All sharks had different lipid compositions, but similar fatty acid and alkyl diol profiles. The high squalene content (50-82% of oil; Figure 5) of all species except *Centroscymnus plunketi* and

Table 5. Comparison of the oil composition of muscle from six oreo species caught off Australia and one species from North Atlantic waters. The data are means and expressed as a percentage of total lipid.

Lipid class		AN (n = 7)	AV (n = 5)	NR (n = 7)	Na (n = 2)	OA (n = 3)	PM (n = 3)	NH (n = 3)
Wax ester	mean	52	10	25	11	9	62	30
	range	22-100	0-34	0-93	2-20	0-27	57-68	5-52
Triacylglycerol	mean	4	3	7	7	30	7	26
	range	0-27	0-17	0-27	0-14	0-66	0-11	14-37
Free fatty acid	mean	11	1	12	11	37	-	19
	range	0-41	0-4	0-42	10-12	0-75	-	3-29
Sterol	mean	4	5	6	5	7	<1	3
	range	0-7	1-9	1-9	4-6	5-9	0-1	1-4
Polar lipid	mean	30	81	67	66	16	30	24
	range	0-71	45-99	1-97	52-80	7-25	27-33	7-52
Total lipid (mg/g wet)								
	mean	29	10	19	7	5	30	16
	range	4-76	4-24	3-56	5-8	4-7	28-31	5-38

Legend:

AN-black oreo (*Allocyttus niger*)
AV-warty oreo (*Allocyttus verrucosus*)
NR-spiky oreo (*Neocyttus rhomboidalis*)
Na-rough oreo (*Neocyttus sp.* A)
OA-oxeye oreo (*Oreosoma atlanticum*)
PM-smooth oreo (*Pseudocyttus maculatus*)
NH- (*Neocyttus helgae*)

Table 6. Comparison of the composition of major fatty acids and fatty alcohols in lipids from the muscle of six oreos caught off Australia and from one oreo from North Atlantic waters. The data are expressed as a percentage of total fatty acids or fatty alcohols. -: not detected.

Component	AN (n = 7)	AV (n = 5)	NR (n = 7)	Na (n = 2)	OA (n = 3)	PM (n = 3)	NH (n = 3)
Fatty acids							
14:0	2.1	0.8	1.2	1.2	0.2	4.2	2.8
16:0	19.5	31.1	29.5	28.3	26.8	12.2	24.4
18:0	3.9	6.4	8.3	5.8	8.2	2.4	5.7
16:1(n-7)	3.6	3	2.1	1.7	1.6	4.5	3.3
18:1(n-9)	21.4	19.9	18.4	13.2	16.5	22.8	13.9
20:1(n-9)	15.4	5.9	5.1	14	3.7	19.5	8.7
22:1(n-11)	4.4	0.8	1.3	4.2	0.4	7.2	5.5
20:4(n-6)	2.1	3.1	4.3	2.4	3.9	1.9	3.3
20:5(n-3)(EPA)	5.1	5.9	4.5	6.4	6.2	4.1	9.7
22:6(n-3)(DHA)	11.7	14.8	16.1	15.6	23.4	9.5	15.7
Others	10.8	8.3	9.2	7.2	9.1	11.7	7
Fatty alcohols							
16:0	7.9	9.1	43.7	1.7	46.6	7.1	4
18:0	4.4	6.7	3.8	0.7	0.5	3.6	4.8
18:1(n-9)	15.8	13.6	6.9	1.6	9.4	10.7	1.3
18:1(n-7)	4.2	10.9	1.7	0.7	0.5	2.9	0.9
20:1(n-9)	38	44.9	24.3	57.3	22.9	40.2	28.8
22:1(n-11)	21.8	12.2	16.3	34.7	19.3	27.8	56.5
22:1(n-9)	4.3	1.2	1.7	2.7	0.7	5.2	2.5
24:1	2.2	0.4	0.9	0.9	-	1.5	1.1
22:6(n-3)(DHA)	1.4	0.9	0.7	-	-	0.9	-

Legend:

AN-black oreo (*Allocyttus niger*)
AV-warty oreo (*Allocyttus verrucosus*)
NR-spiky oreo (*Neocyttus rhomboidalis*)
Na-rough oreo (*Neocyttus sp.* A)
OA-oxeye oreo (*Oreosoma atlanticum*)
PM-smooth oreo (*Pseudocyttus maculatus*)
NH- (*Neocyttus helgae*)

Table 7. Comparison between ratio of 22:1 fatty alcohol to 20:1 alcohol in oreos from Australia and North atlantic waters.

Species/location	Ratio 22:1/20:1
Australia	
Allocyttus niger	0.57
Allocyttus verrucosus	0.27
Neocyttus rhomboidalis	0.67
Neocyttus sp. A	0.61
Oreosoma atlanticum	0.84
Pseudocyttus maculatus	0.69
North Atlantic	
Neocyttus helgae	1.96

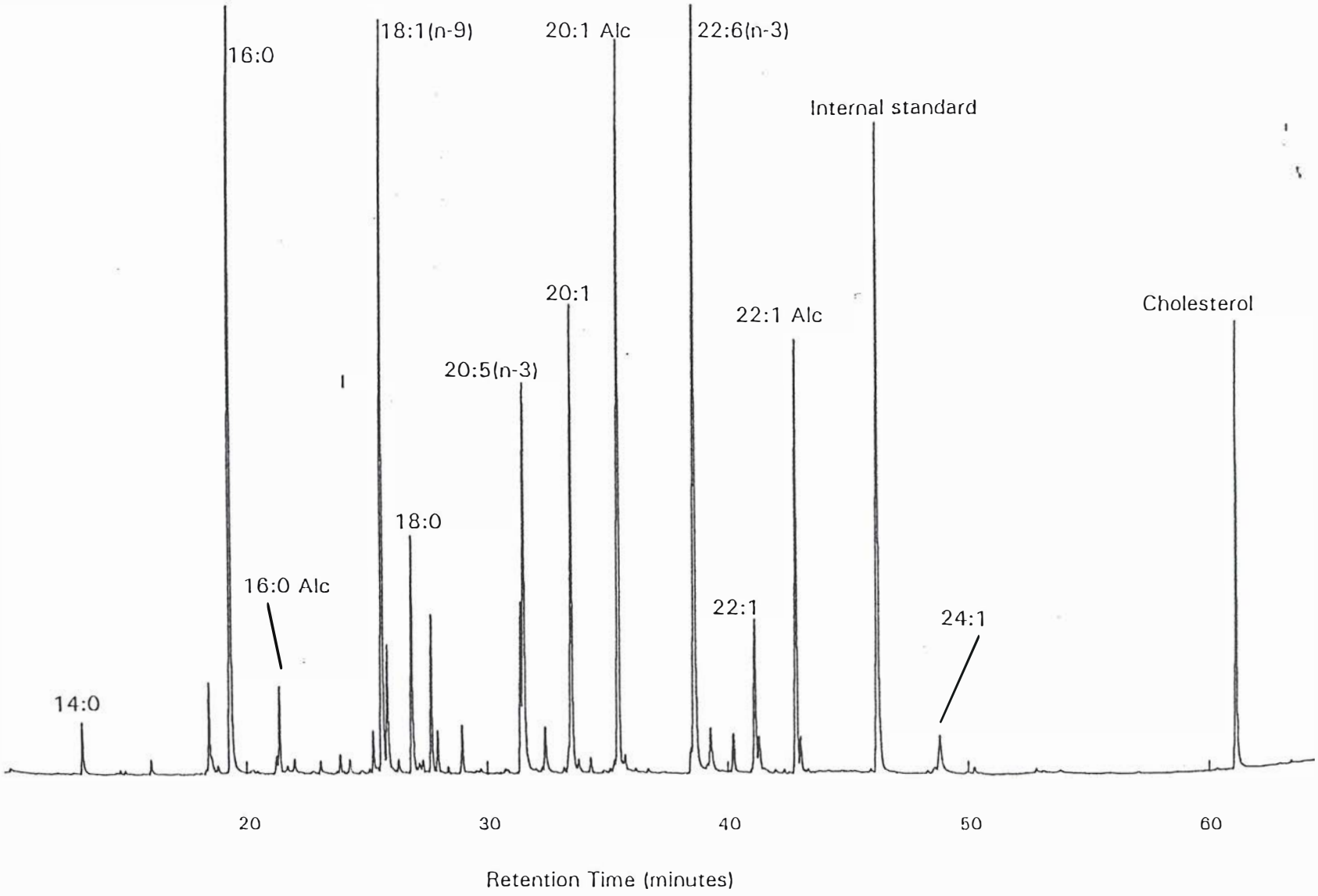

Figure 4. Capillary gas chromatogram of fatty acids (as methyl esters) and alcohols in muscle from *Allocyttus niger*.

Somniosus pacificus suggests that the oil from these deep-sea sharks collected in southern Australian waters will be suitable for industrial uses.

A process to separate squalene from shark liver oils was developed in collaboration with CSIRO Chemicals and Polymers. The process will be used by industry (Squalus and ITL Australia) for the production of squalene for export. The purified export-grade squalene is currently worth approximately $25 per kilogram. It is proposed that Australia can initially export 20-50 tonnes annually (worth $0.5-1.25M); this amount is predicted to increase to 100-200 tonnes (worth $2.5-5M) within several years. At whatever level is achievable, the opportunity exists for the Australian fishing industry to gain a significant portion of the international squalene market.

Laboratory methods were developed to separate the diacylglyceryl ether fraction from shark liver oil. In addition the analytical methods established during the project to routinely characterize shark liver oils were used further to assist Australian companies attempting to purify this fraction for commercial purposes.

4.4 *Omega-3 PUFA rich oils (objective 4)*

Although omega-3 polyunsaturated fatty acids (PUFA) are abundant in most fish oils, no value-added omega-3 PUFA products have been manufactured in Australia. Rather, such products are still imported and crude fish oil is at times exported for value-adding. Unfortunately there have been also episodes where crude Australian fish oil was used as boiler fuel.

It is now well accepted that fish-derived PUFA reduce the risk of coronary heart disease and stroke in humans and also may play a role against a range of other disorders, including arthritis. Capsules of fish oils containing high levels of the essential PUFA are marketed aggressively overseas and have captured a small market in Australia (e.g. MAXEPA and related products). These products typically retail for between $30 and $300 per kilogram (Table 8). Using omega-3 PUFA rich oils derived from Australian species, the potential exists for both import replacement and export of value added products. Representative oil and fatty acid compositional data for capsule products based on imported fish oil are shown in Table 8.

More recently omega-3 oils [also termed (n-3) oils] have been incorporated in other food items; for example, the oils are now added to bread in Scandanavia. The next generation of omega-3 oils contain considerably higher levels of EPA and DHA (approaching 70-80%) and processing of the raw oils is required to achieve these higher levels. The addition of vitamins and other components also may be required to increase product marketability. To date, however, no Australian company has produced and marketed an omega-3 polyunsaturated fatty acid, value-added product. It is our belief that the potential exists, the expertise is available and the timing is right for this to change.

As with the shark liver-derived squalene, initial sales and export of 100-200 tonnes are anticipated if suitable products can be developed; this amount also is predicted to increase within several years. The value of any product will depend on its quality, fishery availability and sustainability and other factors.

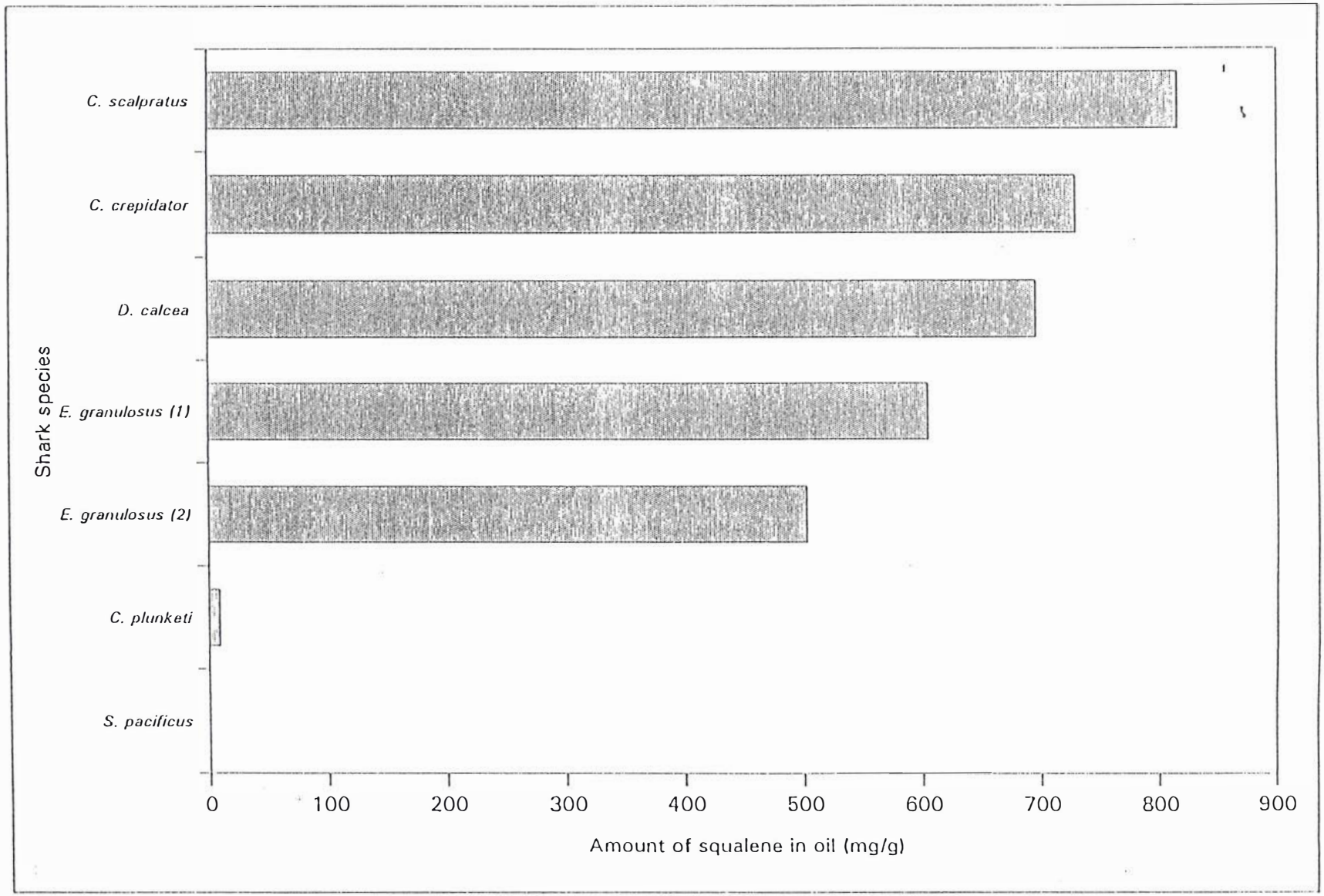

Figure 5. Comparison between squalene content in oils from Australian deep-sea sharks

Table 8. Listing of commercial encapsulated omega-3 and omega-6 polyunsaturated fatty acid oils.

Brand	Name	No. capsules per jar	Price	Oil mass per capsule (mg)	Price per capsule	Price kg oil	EPA capsule	value EPA per kg oil
Cenovis	Evening Primose oil	50	$5.49	550	$0.11	$60.39	-	-
Cenovis	Cod-liver oil	90	$2.98	275	$0.03	$9.11	9%	$0.82
Efamol (Vitaglow)	Fish oil capsules	40	$11.80	1000	$0.30	$295.00	18%	$53.10
Nature's Own	MaxEPA Marine lipid concentrate	50	$11.75	1000	$0.24	$235.00	18%	$42.30
Blackmores	Fish Oil 1000	50	$12.95	1000	$0.26	$259.00	18%	$46.62
Vitaglow	MaxEPA PLUS	40	$13.50	800	$0.34	$270.00	14.4%	$38.88
Nature's Way	OMEGA 3	60	$17.90	1000	$0.30	$298.33	18%	$53.70

The demand for fish oils by the mariculture industry (both in Australia and overseas) also will continue to increase. The economics of fish farming relies on the supply of inexpensive feeds of a suitable quantity and quality, including appropriate oil composition. In particular, EPA and DHA are considered essential for most species reared by the mariculture industry. The quality of any oil required for use in mariculture will be lower than required for health and related products. However, the possible requirement of larger quantities of fish oil by the expanding Australian mariculture industry may be in direct competition to other industries needing sources of omega-3 polyunsaturated fatty acid-rich oils.

Tasmania fortunately has had large catches of jack mackerel; the oil produced commercially from this species contains 25-30% EPA plus DHA (Table 9, Figure 6). When the jack mackerel fishery failed in 1989, oils had to be imported into Australia at considerable cost. At that time, data was not available on possible local replacements. By building the data base on the oil composition of Australian species, industry will be placed better to identify alternative feedstocks and to take advantage of new market opportunities.

4.4.1 Fish oils

In this study, we determined the omega-3 polyunsaturated fatty acid composition for flesh and oils from Australian fish and related samples (Table 9, Figures 4 and 6). The availability of such data is an important prerequisite for the possible production and marketing of omega-3 polyunsaturated fatty acid products by Australian industry.

Many of the fish show potential for use by industries seeking oils rich in EPA and DHA. For example, flesh from the oreos (see also above), and in particular the southern ocean species, Patagonian toothfish (*Dissostichus eleginoides*) and spiny icefish (*Chaenodraco wilsoni*), contain appreciable levels of EPA and DHA (Table 9). Large amounts of the southern ocean species were processed in Hobart during the 1991/1992 summer and, based on lipid compositional data, oil from the flesh of these species would be suitable for use by industry. The commercially-rendered oil obtained from the waste frame, skin and subcutaneous layer of the Patagonian toothfish contained only low levels of omega-3 polyunsaturated fatty acids (Table 9).

Other fish and related oils analysed which contain high levels of EPA and DHA include: (i) oil produced from waste from the salmonid industry, (ii) oil from certain species of zooplankton grown in sewage-treatment ponds, (iii) red bait (*Emmelichthys nitidus*) which is a by-catch associated with the jack mackerel fishery, (iv) mutton bird oil, and (v) oil derived from the southern ocean krill *Euphausia superba* and the local krill *Nyctiphanes australis* (this krill species forms a major part of the diet of jack mackerel and other important commercial species). The major component of the oils is generally triacylgycerol, although the mutton bird (native dialect, Yolla) and oreo oils can contain elevated levels of wax esters (e.g. mutton bird oil contains approximately 60% wax ester). Several of these oils are deep-red in colour due to the presence of dietary-derived carotenoid pigments. This feature also might be of commercial value.

The ratio of EPA to DHA varies considerably between fish species and other samples analysed (Table 9). Similarly, the ratio may also vary based on changes in diet, environment and other factors. In recent times it has been recognized that EPA and DHA play different

Table 9. Major fatty acid composition of selected fish, zooplankton, oils and bacteria.

Fatty acid	Percentage composition												
	FLESH / WHOLE ANIMAL					OIL						BACTERIA	
	Patagonian toothfish	Black oreo	Spiney icefish	Lagoon zooplankton	Euphausia superba	Salmon farm waste oil	Jack mackerel	Orange roughy oil	Red Bait	Patagonian toothfish	Muttonbird oil	Strain NSB31	Strain JSP2W
14:0	2.8	2.7	5.2	1	7.7	4.2	7.3	1.7	9.2	4.7	3.3	0.5	7.5
16:0	16.2	20.4	15.8	11.8	20	15.7	15.7	3.3	11	13.5	5.6	7.5	23.3
18:0	3	3.8	2.4	4.5	1.2	4.2	3.1	0.7	2	3.2	0.8	tr	0.5
16:1(n-7)c	5.4	3.6	7.5	2.7	11.4	5.1	5.1	10.7	2	9.3	15.5	48.4	36.9
18:1(n-9)c	17.7	20.9	8.9	10.5	12.8	16.5	9.9	50.6	5	36.8	30.9	0.8	0.2
18:1(n-7)c	5.9	3.7	7	5.8	10.3	3.5	2.9	5.1	2.3	6.3	4.8	5.6	1.4
20:1(n-9)c	8.3	13.7	0.8	0.6	-	3.3	8.3	17.9	23.6	9.2	2.6	-	-
18:2(n-6)	1.1	1	1.9	10.5	2.3	6.6	1.7	-	1.6	1.2	2.2	tr	-
20:5(n-3) EPA	8.4	5.9	20.6	17.2	19.4	7.1	10.9	-	6.9	-	13	6.3	-
22:6(n-3) DHA	20.3	13.4	24.5	0.8	9.6	15.7	11.5	0.1	9.6	0.9	7.8	-	2.3
Others	10.9	10.9	5.4	34.6	5.3	18.1	23.6	9.9	26.8	14.9	13.5	30.9	27.9
Ratio EPA/DHA	0.41	0.44	0.84	21.50	2.02	0.45	0.95	-	0.72	-	1.67	-	-

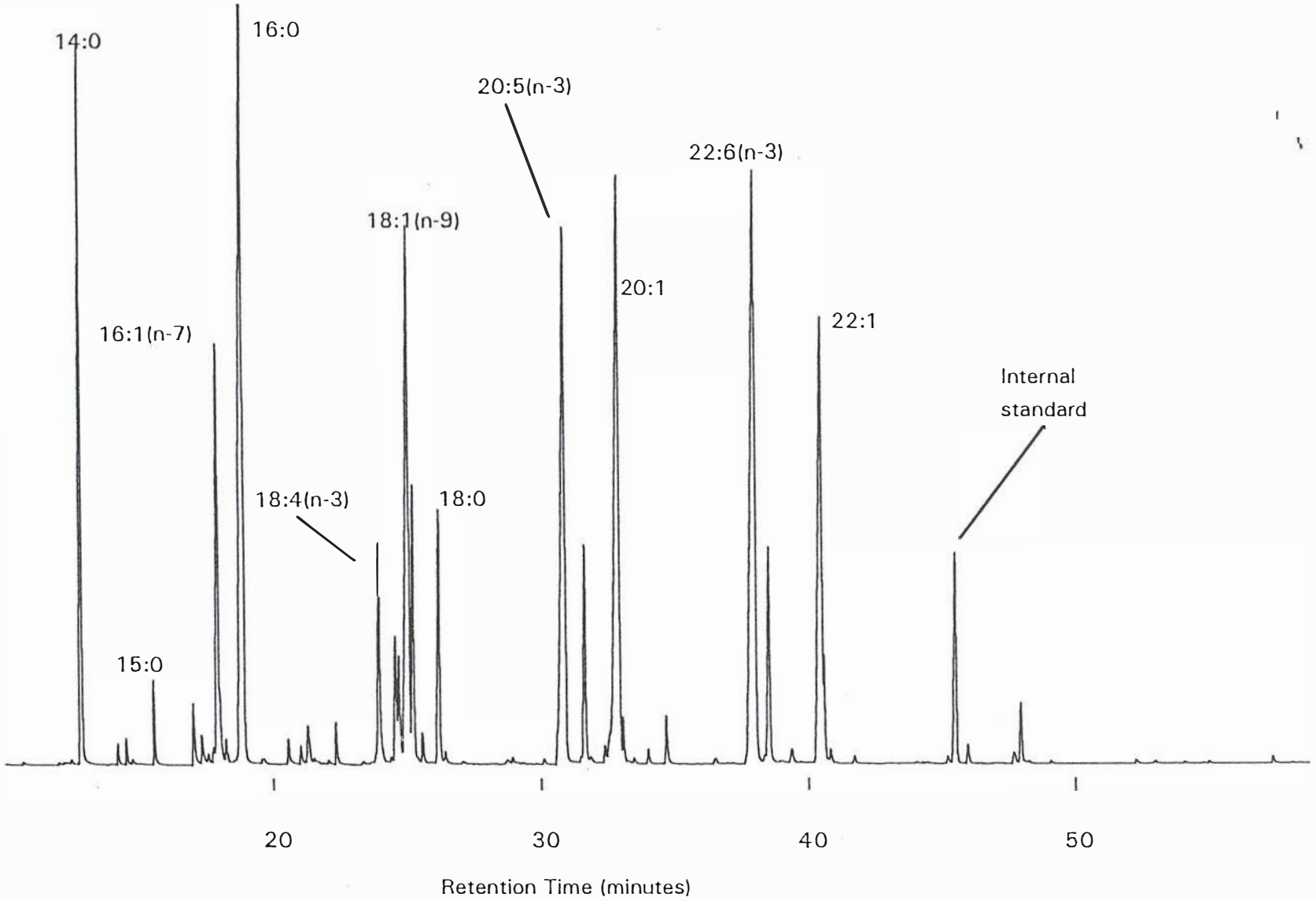

Figure 6. Capillary gas chromatogram of fatty acids (as methyl esters) in jack mackerel oil.

roles in human nutrition. Therefore precise knowledge of the relative levels of these two essential fatty acids may be of use both when targeting oils for further development and as a marketing feature of any Australian products.

To produce marketable Australian omega-3 polyunsaturated fatty acid products, several key issues need to be examined, in particular the sources (reliability, quantity and quality) of the raw oil and processing required to produce competitive products. Such processing could include: deodorizing, separation of lipid classes, enrichment of omega-3 polyunsaturated fatty acids using "winterizing" techniques or through treatment with lipases and resynthesis of triacylglycerols, addition of other components, encapsulation, etc. Several of these steps may be required to produce a higher value-added product. As noted previously, no Australian company has produced and marketed an omega-3 polyunsaturated fatty acid product. The development of any new approaches to the above processes may be patentable, thereby affording partial product protection.

Of the oils described in this report, large quantities of jack mackerel oil are already being produced by Seafish Tasmania at Triabunna on a seasonal basis. The Tasmanian Aborigine Centre (TAC) is in the process of networking the various small operators producing muttonbird oil. The business arm of the Tasmanian aborigine community, Yolla Promotions, has recently successfully launched Yolla as a native food product exclusive to Tasmania, so the potential also exists to commercially produce the mutton bird oil. The precise quantity produced, or the potential to produce many of the other oils described is not presently known. This information will be required if any of the oils are to be used singly or in combination by industry.

4.4.2 Single cell oils (SCOs)

Similar technologies to those needed to produce fish oils can be exploited to isolate and add value to oils from algal and bacterial sources (termed single cell oils, SCOs). Currently a number of groups in Australia are growing the green alga *Dunaliella* for the commercial production of natural carotene. The development of appropriate technology and facilities for the production of value-added marine oils may be transferred to production of fine chemicals, including SCOs, from microalgae and possibly bacteria. Several microalgal species from the brown algal-line produce high levels of (n-3) polyunsaturated fatty acids and others produce arachidonic acid [20:4(n- 6)]. For example, diatoms produce EPA (range 5-20%) with only low levels of DHA present, and certain prymnesiophtye species produce large amounts of both EPA and DHA. Such algae are being used already by the mariculture industry as sources of these fatty acids which are considered essential for the growth and survival of the larval stages of many animal species.

In associated studies, the fatty acid composition of Antarctic bacteria have been examined. Although a limited number of bacteria had been shown to produce PUFA, it was generally thought that most bacteria did not produce EPA and DHA. The knowledge that other organisms produce higher quantities of EPA and DHA at low temperature led to the examination of new strains of bacteria isolated from Antarctic waters. Early results indicate that the proportion of Antarctic strains that produce EPA is considerably higher than found for temperate marine bacteria; EPA levels up to nearly 20% of the total fatty acids have been found (David Nichols, unpublished data). Similarly, a number of strains that produce DHA (up to 3%) also have been isolated (Table 9).

In addition to the presence of EPA in the bacteria, the level of monounsaturated fatty acids was considerably higher than observed for many fish species; the level of saturated fatty acids was lower than in fish. Further research is required to improve the yield of the SCOs. In time, coumpounds made by microalgae and bacteria, such as essential fatty acids, could be obtained and purified using technology developed for the production of value-added products from fish oils.

Further research and development is required on optimizing methods for refining , omega-3 PUFA containing oils, thereby increasing product value. The Australian marine oils industry is still young and fragmented, with many individual players. As such, strong interest for this project has been provided to date by individual companies, however industry funding for the type of research conducted during the FRDC study described in this report has not been available. Funding from FRDC has played a pivotal role in ensuring that the strategic research needed was performed and communicated to Australian industry.

5.0 Recommendations and future research

Research undertaken with FRDC grant 91/77 has provided major impetus to the relatively young Australian marine oils industry, with an emphasis on first, orange roughy oil, then on shark liver oil. Further research is required on other species, particularly those containing omega-3 PUFA, as is more refined work on existing species. The research effort is needed to enable the small businesses involved in this industry to continue to be viable. For Australian industry to achieve an economically sustainable level of value-added products, there is therefore a need to strengthen our knowledge of the oil composition of new commercial and bycatch species and also on species (e.g. various tuna and mackerel) which offer potential for both increased catch and utilization, including oil production and value-adding. Future research in this field will ensure that the economic yields of Australia's fisheries can be increased and environmental concerns minimized with utilization of waste material.

There also is increasing government and public concern for minimizing waste generated by industry, including from aquaculture and seafood processing. This further provides a stimulus for the production of value-added marine oil products from underexploited species and from waste generated by Australian industry.

The composition of orange roughy oil makes it a possible substitute for jojoba oil . These oils are utilised by the cosmetic and pharmaceutical industries after being refined, decolorized and deodorized. This is an area for future development and there is scope to produce value-added high-quality oils in Australia.

Future research is required to meet the needs of Australian industry and the primary objective should be:

- to assist Australian industry develop new marine oil based, value-added products from existing or new fisheries including the bycatch and waste generated by the fishing and related industries. Specific tasks should include:

    - Evaluation of new deep-sea and other shark species for squalene content and oil composition.
    - Determination of the composition of marine oils from underexploited pelagic and other species, with particular reference to:
      (i) identifying optimum sources of omega-3 polyunsaturated fatty acids and other specialty chemicals (e.g. diacylglyceryl ethers from deep-sea and other sharks), and
      (ii) further assisting industry to develop products from these oils and specific oil fractions.
    - Examination and optimization of methods for further refining squalene-containing and omega-3 containing marine oils, thereby increasing product value. The techniques developed should be broad-based and transferable between fish species and the developing field of single cell oils (SCO) derived from microorganisms.

6.0 Acknowledgements

The project involved extensive interaction with Australian industry. We thank the following companies and organizations for provision of samples, ongoing support and encouragement, and for many valuable discussions during the project: BEKU Environmental Products Ltd, Squalus Pty Ltd, Scales Fish and Bait Sales, ITL Australia (Chemicals), Clover Corporation, Saltas, Tassal, Aquatas, Seafish Tasmania, Zootech, Trident Seafoods, Tas Crays, AMRAD, ABL & MOR Pty Ltd, Allisons Laboratories, Harmex, Sunshine Trading Co., R P Scherer, Unichema, Deer Horn of Australia, Tritech Chemicals, Queensland IFRI, CSIRO Division of Chemicals and Polymers, CSIRO Dairy Research Laboratory, CSIRO Division of Human Nutrition, Departments of Agricultural Science, Biochemistry and Zoology at the University of Tasmania, Antarctic CRC, Deakin University, The Australian Antarctic Division, the officers and crew of *Aurora Australis,* NSW Fisheries, Australian Institute of Marine Science, The Tasmanian Aborigine Centre, Chemistry in Australia, New Zealand DSIR, The Kyung Hee University in Korea.

APPENDIX

Marine oil Publications

(Manuscripts are attached to the report; associated manuscripts are available on request and reports are held by the CSIRO Marine Laboratories)

Elliott, N., Skerratt, J. and Nichols, P. (1990) Orange roughy oil proves it worth. **Australian Fisheries** August, 32-33.

Daintith, M. and Nichols, P. D. (1990) Nutritional aspects of the rotifer *Brachionus plicatilus*. **Austasia Aquaculture Magazine**, **4**, 16-17.

Volkman, J. K. and Nichols, P. D. (1991) Applications of thin-layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. **J. Planar Chromatography-Modern TLC 4**, 19-26.

Nichols, P. D., Nichols, D. S. and Volkman J. K. (1993) Recent developments with marine oil products in Australia. **Chemistry in Australia** 60: 336-340.

Nichols, D. S., Williams, R., Dunstan, G. A., Nichols, P. D. and Volkman, J. K. (1994) Fatty acid composition of Antarctic and temperate fish of commercial interest. **Comp. Biochem. Physiol., 107B,** 357-363.

Nichols, P. D., Nichols, D. S. and Bakes, M. J. Developments with Marine Oil Products in Australia. **Int. News on Fats, Oils and Related Materials**. (in press).

Bakes, M. J. and Nichols, P. D. Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. **Comp. Biochem. Physiol.** (manuscript submitted).

Woodward, D. R., Riley, M., Buick, D., Nichols, P. D. and Nichols, D. S. Nutritional analysis of the flesh and oil of the Tasmanian mutton bird *Puffinus tenuirostris*: a useful source of omega-3 fatty acids. **Medical J. Australia** (manuscript submitted).

Two other manuscripts are in preparation on the lipid composition of oreos and orange roughy.

Associated papers

Nichols, D. S., Nichols, P. D. and McMeekin, T. A. (1992) Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* strain ACAM 342. **FEMS Microbiology Letters** 98:117-122.

Virtue, P., Nichols, P. D., Nicol, S., McMinn, A. and Sikes, L. (1993) The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii*. **Antarctic Science 5**: 161-167.

Nichols, D. S., Nichols, P. D. and McMeekin, T. A. (1993) Polyunsaturated fatty acids in Antarctic bacteria. **Antarctic Science 5**: 149-160.

Virtue, P., Nicol, S. and Nichols, P. D. (1993) Changes in the digestive gland of *Euphausia superba* Dana during short term starvation. Lipid class, fatty acid and sterol content and composition. **Mar. Biol., 117**, 441-448.

McMeekin, T. A., Nichols, P. D., Nichols, D. S., Juhasz, A. J. and Franzmann, P. D. Biology and biotechnological potential of halotolerant bacteria from Antarctic saline lakes. **Experimentia** (in press).

Nichols, D. S., Nichols, P. D. and McMeekin, T. A. Optimization of polyunsaturated fatty acid growth by *Shewanella putrefaciens* strain ACAM 342 by alteration of growth conditions **J. Gen. Microbiol.** (in press).

Marine oil reports

Nichols, P. D., Dunstan, G. A. and Watson, M. (1990) Lipid and fatty acid composition of Huwamol Royal Red and Golden King Fish oils. Report 90-SHW1. Report prepared for S. H. Wellington Pty. Ltd.

Nichols, P. D. and Elliott, N. (1990) Lipid, fatty acid and fatty alcohol composition of Wini orange roughy oil. Report 90-WINI1. Report prepared for Wini Pty. Ltd.

Nichols, P. D. (1991) Lipid, fatty acid and fatty alcohol composition of Trident Dory oil. Report 91-Trident1. Report prepared for Trident Seafoods Pty Ltd.

Nichols, P. D. (1991) Analysis of a transmethylated fatty acid and fatty alcohol product derived from orange roughy oil during laboratory trials. Report 91-Ivey1. Report prepared for John Ivey.

Nichols, P. D. (1991) Oil composition of products under consideration for use in the BEKU degreaser. I. New Zealand orange roughy oil and II.Tas Crays Pty. Ltd. compost by-product oil. Report 91-BEKU1. Report prepared for BEKU Ltd.

Nichols, P. D. (1991) Oil composition and market potential of Trident shark liver oil. Report 91-Trident2. Report prepared for Trident Seafoods Pty Ltd.

Nichols, P. D. (1991) Oil composition of products under consideration for use in the BEKU degreaser. III. Deodorized Tas Crays by-product oil. Report 91-BEKU2. Report prepared for BEKU Ltd.

Nichols, P. D. (1991) The composition of new Trident oils produced from: I whole shark and II Salmon farm waste. Report 91-Trident3. Report prepared for Trident Seafoods Pty Ltd.

Nichols, P. D. (1991) Composition of marine oils under consideration for use in the BEKU degreaser and other products. I. *Hopolos* oil and II. *Dorolos* oil. Report 91- BEKU3. Report prepared for BEKU Ltd.

Nichols, P. D. (1991) Composition of marine oils under consideration for use in the BEKU degreaser and other products. I. Deodorised Trident orange roughy oil and II. Deodorised Tas Crays Pty Ltd compost by-product oil. Report 91-BEKU4. Report prepared for BEKU Ltd.

Nichols, P. D. (1992) Report on the use of BEKU environmental products during 1991/92 Antarctic field season. Report 92-BEKU1. Report prepared for BEKU Ltd.

Nichols, D. S., Nichols, P. D. and Volkman, J. K. (1992) Squalene content and lipid class composition of Endeavour shark liver oil. Report 92-SWS1. Report prepared for Sydney Wide Seafood.

Nichols, D. S. and Nichols, P. D. (1992) Lipid, fatty acid and fatty alcohol composition of deodorized orange roughy and orange roughy/dory oils. Report 92-BEKU2. Report prepared for BEKU Environmental Products Ltd.

Nichols, D. S. and Nichols, P. D. (1992) Composition of a mixed oil sample produced during fishing trials conducted by a Russian vessel in the Southern Ocean. Report 92-BEKU3. Report prepared for BEKU Environmental Products Ltd.

Dunstan, G. A. and Nichols, P. D. (1992) Lipid class composition of soft-gel capsules. Report 92-DSIR2. Report prepared for DSIR, Seafood Research Laboratory.

Nichols, D. S., Dunstan, G. A. and Nichols, P. D. (1992) Oil, fatty acid content and composition and proximate composition of daphnids and mixed zooplankton obtained using the Frish polyculture harvester. Report 92-Frish1. Report prepared for Frish Pty Ltd.

Nichols, D. S. and Nichols, P. D. (1992) Composition of a mixed oil sample produced from fishing trials conducted by a Russian vessel in the Southern Ocean during March and April 1992. Report 92-BEKU4. Report prepared for BEKU Environmental Products Ltd.

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ORANGE ROUGHY OIL PROVES ITS WORTH

CSIRO scientists Nick Elliott, Jenny Skerratt and Peter Nichols report that waste from the processing of orange roughy could be creating dollars rather than environmental problems.

There was a public outcry in Tasmania in 1989 at the local environmental problems caused by the dumping of waste from the processing of orange roughy. Yet this waste represents a considerable resource of material for oil and meal production if the fishery continues to harvest at its current high levels.

While good quality meals can find a ready use in animal feeds, in both domestic and export markets, local fish oil production is not yet large and companies currently import fish oils from New Zealand. Recent work by CSIRO, however, indicates that the Australian orange roughy catch is a potential local source of fish oil.

Orange roughy oil

A significant attribute of orange roughy is that many parts of its body contain copious quantities of oil, which improves buoyancy and/or acts as an energy reserve (Body, 1982). Approximately 18 per cent of the whole fish consists of oil (Buisson et al., 1982). Unlike the oil of most other commercial fish, orange roughy oil is composed almost entirely of wax esters, making it a possible substitute for sperm whale and jojoba oils (Buisson et al., 1982). About 80 per cent of this oil is located in the waste products from filleting — the head, swim bladder, frame and skin.

It is estimated that processed fillets constitute approximately 30 per cent of the Australian catch (Thrower and Bremner, 1987), with the remainder being waste material. This means that of a catch of 10 000 tonnes, over 1800 tonnes of oil would be discarded. Based on an estimate of $1.00 per kilogram of oil, that represents a potential $1.8 million turned instead into an environmental headache.

CSIRO's study

CSIRO compared the composition of oil from Australian-caught specimens of orange roughy with reported data on specimens caught off New Zealand and Britain.

The oil was extracted from the swim bladders of 10 specimens and the muscles (fillets) of two. The swim bladder was selected as it is reported to contain over 60 per cent lipid with a composition representative of other tissues (Grigor et al., 1983). The swim bladders were from fish caught during the 1988 CSIRO trawl surveys of the southeastern waters of Australia (Elliott and Bulman, 1989) and the muscle samples were from specimens caught off the east coast of Tasmania during the 1989 CSIRO trawl survey. All samples were frozen at sea.

The composition of the wax esters, fatty alcohols and fatty acids in the samples were analysed at the CSIRO Division of Oceanography laboratories, using facilities established specifically for studies of marine lipids.

The results

Around 97 per cent of the swim bladder oil in the Australian-caught orange roughy was present as wax ester, with about three per cent as phospholipids and ?0.5 per cent as triacylglycerols. This value is comparable to that reported for specimens caught off New Zealand (95 per cent) and Britain (90 per cent). Likewise the composition of the major fatty acids and fatty alcohols is similar for the three regions (Table 1).

Variations in the proportions of the individual fatty acids and fatty alcohols, with the size of the fish or the place of capture, may prove significant with analysis of more samples and further statistical analysis. For example, the proportions of the fatty acids EPA (eicosapentaenoic acid, 20:5 [n-3]) and DHA (docosahexaenoic acid, 22:6 [n-3]) were 4.6 and 4.8 per cent respectively in the oil from the swim bladders of 21 cm fish, but both were only 1.4 per cent in the oil from the swim bladders of 37 cm fish. Similar differences were observed in the fatty acid composition of the oil derived from the muscle of 21 cm and 37 cm specimens (Table 2).

Oil from the muscle and swim bladder of New Zealand-caught specimens (Grigor et al., 1983) had less EPA and DHA fatty acids than the Australian-caught specimens. While only two muscle samples from different-sized specimens were analysed in this CSIRO study, the results indicate the need for further investigation of size- (and therefore age-) related differ-

Table 1

Comparison of the composition of major fatty acids and fatty alcohols in wax esters from swim bladder oil of orange roughy caught off Australia, New Zealand and Britain.

The data are expressed as a percentage of total fatty acids or fatty alcohols.

Constituent**	Australia[1]		New Zealand[2]		Britain[3]	
	alcohol	acid	alcohol	acid	alcohol	acid
14.0	3.8	1.8	1.7	1.7	2.3	0.8
16.0	30.8	2.3	24.2	2.0	24.0	1.6
16:1(n-7c*)	0.6	12.3	3.6	13.0	2.1	10.9
18:0	7.6	0.9	7.3	0.8	4.6	0.1
18:1(n-9c*)	15.7	46.2	17.4	54.0	17.0	50.8
20:1(n-9c*)	23.5	17.0	26.7	17.1	22.0	18.3
22:1(n-11c*)	8.7	4.7	16.0	7.9	28.0	14.6
Others	9.3	14.8	3.1	3.5	—	2.9

1. This study (mean of five fish, all 37 cm, 1.75 to 2.1 kg);
2. Ref. Grigor et al. 1983 (mean of six fish, 0.5 to 1.9 kg);
3. Ref. Sargent et al., 1983 (20 cm, 0.3 kg).
* cis geometry, this study only.

**Fatty acid and fatty alcohol nomenclature: Fatty acids and fatty alcohols are designated as total number of carbon atoms: number of double-bonds followed by the position of the double-bond from the aliphatic end of the molecule; e.g. 20:5(n-3) means that there are 20 carbon atoms in the alkyl chain comprising the molecule, with five double (unsaturated) bonds in the n-3 series, the first double bond being three carbon atoms from the aliphatic (methyl) end of the molecule. The suffixes 'c' and 't' indicate cis and trans geometry respectively.

Table 2

Comparison of fatty acid and fatty alcohol composition of the wax ester extracted from the muscle of a 21 cm and a 37 cm orange roughy caught off the east coast of Tasmania.

The data are expressed as a percentage of total fatty acids or fatty alcohols.

*Constituent***	*21 cm specimen*		*37 cm specimen*	
	alcohol	*acid*	*alcohol*	*acid*
14:0	3.5	1.3	1.9	0.8
16:0	36.7	6.3	28.3	2.9
16:1(n-7c)	0.2	11.5	0.3	8.8
18:0	8.9	0.8	9.2	0.9
18:1(n-9c)	14.4	40.3	14.4	51.9
20:1(n-9c)	17.1	10.3	22.5	17.9
20:5(n-3)	nd	4.5	nd	0.6
22:1(n-11c)	9.9	3.0	12.6	5.0
22:6(n-3)	nd	11.3	nd	2.8
Others	9.3	10.7	10.8	8.4

nd, not detected

**see note to Table 1

ences, as well as geographic differences in the amount of individual fatty acids and fatty alcohols.

The reason for such differences is not known, but it may reflect the composition of the diet and/or the physiological state of the fish at time of capture. One origin of the components of the oil may be via the food chain, and the diet of the orange roughy changes as the animal grows (Rosecchi et al., 1988; Bulman, C. personal communication).

The relative abundance of the (nutritionally) essential fatty acids in the muscle of a commercial-size specimen (37 cm) was very low (EPA = 0.6 per cent; DHA = 2.8 per cent) compared with values reported for other commercial Australian fish (for example trevalla, EPA = 4.7 per cent, DHA = 30 per cent; Bremner et al., 1989). Orange roughy fillets are therefore not a good source of these 'beneficial oils'. The high wax ester content is also of concern, as wax esters are not easily digested. However, results from controlled feeding trials on rats and pigs in New Zealand suggest that 'normal' consumption of orange roughy by humans should not lead to any health problems (James and Treloar, 1984; James et al., 1986).

There was a further outcome from this study. Because the monounsaturated alkyl chains — which are very abundant in orange roughy oil — were fully identified, the distinctive profile obtained will make it possible to identify the oil from orange roughy waste in environmental studies (Nichols et al, 1989).

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Editor's note: Methods of handling and processing oil and fish meal from orange roughy waste were reviewed by Stephen Thrower and Alan Bremner in their article 'Orange roughy — a guide to handling, chilling and processing' in the November 1987 issue of *Australian Fisheries*.

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NATIONAL KEY CENTRE FOR TEACHING & RESEARCH IN AQUACULTURE

NUTRITIONAL ASPECTS OF THE ROTIFER *Brachionus plicatilis*

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The rotifer *Brachionus plicatilis* is widely used as a live feed for rearing fish and crustacean larvae, especially in marine or brackish water culture. It is a suitable size, is acceptable and digestible, and mass production is possible (Battaglene and Talbot, 1989).

The nutritional value of the rotifer to the target organism includes lipids, amino acids, carbohydrates, vitamins and minerals. Of special importance to marine larvae is the content of w3 essential fatty acids (EFA) in the rotifer.

This report looks at some aspects of incorporation of EFA in the rotifer and is based on joint research undertaken by the CSIRO Division of Oceanography (Hobart) and the National Key Centre for Aquaculture, Launceston (see Nichols et al., 1989).

Rotifers are commonly raised in hatcheries using a combination of yeast and microalgae, and the lipid profile of this food is reflected in the rotifer. The w3. EFA are virtually absent in yeast resulting in unfavourably low levels of EFA in rotifers fed yeast only.

Some microalga do contain significant levels of EFA but the small amounts used in these combination diets may again result in low levels of EFA in the rotifer. Several techniques are used to raise the level of EFA in the rotifer prior to feeding to the target organism including feeding with a microalga rich in EFA, enriching with microencapsulated feeds or commercially available marine lipid mixtures.

TIMING IS CRUCIAL

This study was undertaken to assess how quickly EFA are incorporated in the rotifer when fed the microalga *Pavlova lutheri*. This alga has been used by overseas workers and is now widely used in Australian mariculture operations because of its high EFA content. The time required for incorporation of the EFA and other essential components is an important aspect of hatchery management. Longer time intervals require more microalgae to feed the rotifers and this means greater expense. Too short a time, however may result in undesirably low levels of EFA.

Rotifers previously fed yeast for two weeks were resuspended in filtered sea water and fed *P. lutheri*. Samples of the rotifer were taken at 0, 3, 6, 12, 24 and 48 hours, and one additional sample was taken after starving for a further 48 hours. These samples were filtered, rinsed and stored frozen in dry ice prior to delivery to the CSIRO Marine Laboratories.

At CSIRO the rotifers were dissolved in a mixture of chloroform and methanol to extract the lipids then analysed, using Thin Layer Chromatography - Flame Ionisation Detection, Gas Chromotography (GC) and GC - Mass Spectrometry. These exacting techniques can be used to provide qualitative and quantitative analysis of the complete lipid profile of organisms.

Results indicated that the lipid content of rotifers (per animal basis) increased when fed *P. lutheri* and reached a peak after 12 to 24 hours. Rotifers starved for 48 hours showed a four-fold decrease in lipid content.

The percentage composition of the EFA 20:5 w3 (eicosapentaenoic acid, EPA) and 22:6 w3 (docosahexaenoic acid, DHA) is shown in Figure 1. EPA and DHA have been shown to be of crucial importance in growth and survival of marine larvae. We observed a significant incorporation of these EFA by the rotifer after 3 hours feeding with *P. lutheri* and a steadily rising incorporation to the termination of the trial at 48 hours.

Hatchery management decisions can be made on the basis of studies like these. As demonstrated here, feeding *B. plicatilis* with *P. lutheri* for 12-24 hours will result in significant incorporation of EFA and total lipid. Starved rotifers show a marked decrease in total lipid and percentage EFA. Starved rotifers can result from overfeeding in larval rearing tanks, with uneaten rotifers starving before they are subsequently consumed.

Future studies will look at alternative diets to replace microalgae and the cost of these diets and microalgae. In addition, we will examine the efficiency of the transfer of EFA from either algae or other prepared diets through the food chain (rotifers and zooplankton) to the larvae of marine fish.

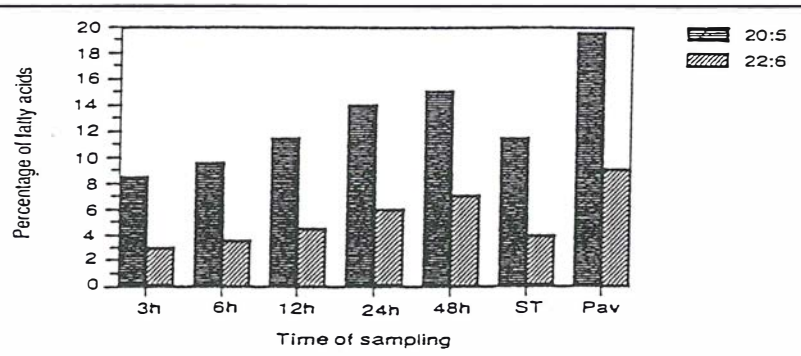

Figure 1. Relative amounts or 20:5w3 and 22:6w3 in the rotifer *Brachionus plicatilis* fed on the microalga *Pavlova lutheri*. Sampling times are as shown. ST, *Pavlova*-fed then starved; Pav, *P. lutheri*.
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LIPID ANALYSIS FOR AQUACULTURE

The CSIRO Marine Laboratories in Hobart offer a lipid analysis service for aquaculture. Details may be obtained from John Volkman or Peter Nichols, CSIRO Division of Oceanography, CSIRO, G.P.O. Box 1538, Hobart, Tasmania, 7001, Australia.

ACKNOWLEDGEMENTS

We thank J.K. Volkman, D.G. Holdsworth and S. Allanson for valuable comments and laboratory assistance.

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TROPICAL AQUACULTURE

By Colin Shelley, Director of Aquaculture, Department of Primary Industry & Fisheries, GPO Box 990, Darwin NT 0801, Australia.

BATTLING BARRAMUNDI FARMERS

Australian barramundi farmers may be forgiven for believing that their newly found industry is suffering more than its fair share of growing pains.

Problems with the quality of both larvae and feed, a general lack of co-ordination between industry and government, coupled with disease outbreaks, have made it difficult to maintain financially viable operations. However, indications suggest that the early years of this decade will see the farmers starting to reap the benefits of the late 1980's research and development.

Several hatcheries are now supplying barramundi fingerlings. Increased competition will undoubtedly lead to improved fingerling quality and competitive pricing. Most hatcheries have relied heavily on fertilised eggs, hand stripped from wild Weipa barramundi. However, progress with the use of spawning induction techniques by government hatcheries in the Northern Territory and Queensland theoretically means that fertilised eggs and fingerlings can now be produced to order from captive barramundi broodstock throughout the breeding season.

Whilst hatchery fingerling capacity needs to be steadily expanded over the next few years, it is the number of ponds and cages devoted to barramundi grow-out that must increase if barramundi production is to reach an estimated 1,000 tonnes per annum by 1994-95, as predicted by Dos in his status report on Australian aquaculture (see February's issue of AAM, Vol 4, No 7, pages 2-12).

New feed mills opening up in Australia will hopefully increase the quality of feed available to grow-out farmers, and decrease the cost. Many farmers are now insisting on getting feed delivered in freezer or chilled containers to minimise vitamin losses and are storing feed on site in freezer facilities.

Feed mills are listening to farmers and efforts are being made to produce feeds that don't sink rapidly, which is especially important for cage culture operations.

Barramundi farmers are going to face competition from Thailand, Taiwan, Vietnam and probably other Asian nations exporting barramundi products to Australia. As an industry in Australia, barramundi aquaculture must aim for the highest quality of product, continuity of supply and examine all avenues of product handling and processing available to find new markets and expand existing ones.

If you are involved with the barramundi farming business as a farmer, hatchery operator, scientist or investor then the new quarterly newsletter **Barramundi Aquaculture** is for you. The first copy was due out late February, early March 1990, so if you have not received your copy please contact me on (089) 89-4321 or fax (089) 81-1475 or write to the address at the head of this column.

Next month :

In next month's issue Colin will cover the heating options for the tropical and sub-tropical winter. Any requests for articles to be included in this column can be sent to Colin.

Applications of Thin Layer Chromatography-Flame Ionization Detection to the Analysis of Lipids and Pollutants in Marine and Environmental Samples

John K. Volkman* and Peter D. Nichols

Key Words:

Marine lipids
Pollutants
Microalgae
TLC-FID
Hydrocarbons
Sewage
Mariculture

Summary

Applications of thin layer chromatography-flame ionization detection (TLC-FID) to the analysis of natural lipids and pollutant compounds in seawater, sediments, microalgae and marine animals are presented. The method combines good sensitivity, ease of use, and the ability to resolve most of the compound classes of interest in marine samples. There are, however, still some difficulties with quantitation, particularly in the choice of internal standard and calibration of the FID for different lipid classes. New applications where the TLC-FID technique offers advantages over, or complements, existing analytical techniques include: the quantitation of petroleum-derived hydrocarbons in environmental samples; the analysis of marine oils; screening lipid extracts for new compound classes; determination of the abundance of lipid classes in natural and polluted sediments and seawater; the analysis of lipids in mariculture feedstocks; tracing the uptake of lipids by fish, molluscs and crustaceans raised in hatcheries; and determination of 5β-stanols in sewage-derived materials.

1 Introduction

The combination of thin layer chromatography with a sensitive flame ionization detector (TLC-FID) had its genesis in the late 1960s and early 1970s with the pioneering work of *Padley* [1] and *Szakasits et al.* [2]; see the book by *Ranný* [3] for a review. These ideas were then developed further by *Okumura* and *Kadano* in Japan [4, 5] who produced reusable TLC rods with a sintered silica gel layer which are now commercially available as Chromarods. Although the first TLC-FID system was developed by Unilever, it was the independent work of the Iatron Laboratories in Tokyo and the Shionogi Company of Osaka that led to the first commercially available instrument, now known as the Iatroscan TLC-FID analyzer [6, 7]. Separations are carried out using conventional TLC techniques on rods of quartz coated with a layer of silica gel or alumina. After development, the rods are dried and scanned through the FID of the Iatroscan analyzer.

This new technique has found widespread application in a variety of fields. It is particularly suited to the analysis of samples where the amounts may be small (submilligram), where high sample throughput is desired (10 rods can be used simultaneously), or where samples are too involatile or polar for capillary gas chromatographic analysis. TLC-FID has proved to be invaluable for use in industry, for the analysis of fats and oils in the medical, biochemical and pharmaceutical fields, and for the analysis of marine lipids in organisms, sediments and seawater. A textbook on TLC-FID principles and applications is available [3].

In this paper, we describe some examples of the use of TLC-FID in our laboratories to study the lipids and other organic constituents of a variety of marine samples. Our experience is based on the use of a Mark III Iatroscan over a period of almost 10 years. Most of these applications can be directly transferred to the more recent Mark IV and V instruments, although it should be noted that some aspects of quantitation are less of a problem with the newer models.

2 Experimental

Lipids were extracted from samples using a modification of the *Bligh* and *Dyer* [8] solvent system designed for extraction of fish lipids. The extracts were analyzed for lipid class composition by TLC-FID, while data on individual components were obtained by capillary gas chromatography with FID and mass spectrometric detectors. Typical operating conditions for the Iatroscan Mark III TH-10 TLC-FID analyzer were de-

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scribed by *Volkman et al.* [9]. The FID was operated with a hydrogen flow rate of 160 ml min^{-1} and an air flow rate of 2000 ml min^{-1}. The hydrogen was instrument grade supplied from cylinders, but the air was obtained from a simple aquarium pump. The rods were scanned at setting 4, which corresponds to a speed of 0.42 cm s^{-1}.

3 Results and Discussion

3.1 Practical Considerations

3.1.1 Application of Extract to the Rods

Extracts are applied ("spotted") at the origin of the rod using either a microliter syringe or micropipet, depending on the preference of the operator. Micropipets (Drummond Microcaps or equivalent) of 0.5 or 1.0 μl capacity are simple to use and give reproducible results after some practice. The syringe is more practical when the volume to be applied is 5 μl or more, although care must be taken not to scratch the silica surface and to ensure that small volumes are spotted with sufficient time between applications to minimize band spreading. Another approach is to develop the rods briefly in a solvent system more polar than that to be used for the subsequent analysis in order to focus the compounds at the origin before development [11]. Improved mechanical applicators have been developed [10], and commercial applicators are also available.

A set of 10 Chromarods is held in a metal frame so that 10 samples may be developed simultaneously and scanned sequentially. In practice, however, it is common to have authentic standards on 1 or more rods and to analyze each sample in duplicate or triplicate so that analysis of 3 – 4 samples at the one time is more common. After sample application, the rods are allowed to dry in air for a few minutes and they are then placed in a glass tank, lined with preextracted filter paper, containing the desired solvent system. As in conventional TLC, it is very important to ensure that the atmosphere in the tank is equilibrated with the solvents.

3.1.2 Solvent Systems

A variety of solvent systems can be used, depending on the specific application, but we have found that hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*) is a good general-purpose system for resolving hydrocarbons, triacylglycerols, free fatty acids, alcohols and sterols from more polar lipids [9]. Since this system does not separate hydrocarbons from alkyl (wax) and sterol esters, we also use hexane-diethyl ether (96 + 4; *v/v*) when the latter compounds are thought to be present. A useful system for separating polar lipids is chloroform-methanol-water (80 + 15 + 2; *v/v*). More complicated development schemes have been described by *Parrish* [12] and *Nichols et al.* [13]. These use multiple developments and partial scanning of the rods through the FID using the stop-scan facility of the Iatroscan. Different solvent systems of increasing polarity were used sequentially to obtain 3 – 4 chromatograms per rod. In the scheme used by *Parrish* [12], hydrocarbons, wax esters, methyl esters and ketones were separated by double development with hexane-diethyl ether-formic acid (99 + 1 + 0.05; *v/v*); triacylglycerols, free fatty acids, fatty alcohols, sterols and diglycerides were separated using hexane-diethyl ether-formic acid (80 + 20 + 0.1; *v/v*), and various polar lipids were separated using two double developments, first with acetone and then with methylene chloride-methanol-water (5 + 4 + 1; *v/v*). This technique is labor intensive and probably best suited for specific applications rather than routine analyses. After development, the Chromarods are oven-dried for 5 min at 80 – 100 °C and then immediately analyzed using the Iatroscan. Each scan takes only 30 seconds, so there is considerable potential for analyzing large numbers of samples in a day. Several sets of rods can be developed at the same time and scanned within a few minutes of each other. A single Iatroscan analyser can thus usually satisfy the needs of most analytical research laboratories.

One advantage of the Chromarod system is the ability to alter the form of the silica for specific applications, for example by impregnating the rods with oxalic acid for phospholipids [14], or with boric acid or silver nitrate to resolve isomeric [15] or unsaturated components [16], respectively. The quality of the rods and the way that they are handled greatly affects the resolution obtained. It is important to minimize the possibility of the rods adsorbing atmospheric contaminants. Immediately prior to use, the rods are activated and cleaned of contaminants by scanning them in the FID. They may be used for more than 100 analyses, provided that residues remaining after scanning are burned off or removed by soaking in strong acid (*e.g.* 35% nitric acid or chromic acid) followed by distilled water rinses. It is preferable to store rods in a desiccator when not in use.

3.1.3 Detection and Calibration

TLC-FID is 2 – 3 orders of magnitude less sensitive than GC-FID. It compares favorably, however, with most HPLC detectors commonly used for lipid analysis, such as UV, refractive index, or the evaporative light scattering mass detector [17]. Lipid amounts less than 0.2 μg are difficult to measure accurately by TLC-FID. On the Mark III instrument, the FID response is approximately linear up to several tens of μg [9, 12], but at low loadings (< 2 μg), a curved response may be observed, often with a non-zero abscissa. Thus, the response of the FID has to be calibrated using external standards which cover the range of concentrations found in the sample. We prefer to work in the range 2 – 5 μg for each compound class. Different lipid classes have different FID responses, and hence different calibration curves [9, 12]. Hydrogenation of the lipid extract is reported to give higher responses for commonly occurring lipid classes [18].

Quantitative aspects of the Iatroscan TLC-FID have been the subject of much discussion [9, 12, 19, 20]. Results may be reproducible to ± 8% [9], although accuracy depends on the choice of standards. Natural lipid classes contain a great number of different compounds varying in chain-length and degree of unsaturation. These will have slightly different re-

sponses, and thus a single standard can only ever give an approximate value. This factor is particularly significant when polar compounds are measured, since their responses vary greatly.

Another approach to quantitation is to include internal standards. While this overcomes variations induced by errors in sample application, it can lead to errors at low loadings where the detector response is non-linear. For most complex lipid mixtures, internal standards are not feasible since their is little space in the chromatogram for an additional compound. Some authors have used a long-chain ketone, which elutes between triacylglycerols and wax esters, as an internal standard [21]. Long-chain ketones also occur naturally in some samples, however, (*e.g.* certain species of microalgae from the class Prymnesiophyceae [22]), and tailing of the wax ester peak may interfere with the measurement of the ketone [12]. Our experience shows that there is often more space between free fatty acids and sterols, so an internal standard which elutes in this region may be of more widespread use.

3.1.4 Data Handling

In our earlier studies, the FID output was recorded on a YEW 3056 2-pen recorder and an electronic recording integrator (Chromatopac). More recently we have used a commercial data acquisition system (DAPA Scientific Pty Ltd, Kalamunda, Western Australia) running on an IBM-compatible personal computer. Examples of both outputs are shown in this paper. Several integrating and data reporting packages are now available, although some are not well suited for this application. The TLC signal from the Iatroscan drops below zero between each scan, which can make it difficult for the integrator to establish the true baseline. A system which allows postrun manual setting of baseline and integration points is desirable.

3.2 Applications

3.2.1 Lipids in Seawater

The identities and concentrations of the major lipid classes in seawater are readily determined by TLC-FID. Applications are described in a number of papers [9, 12, 21, 23–27]. A useful solvent system for lipid separation on silica S-III rods is hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). This resolves hydrocarbons, triacylglycerols, free fatty acids, and sterols from more polar lipids [9]. Polar lipids generally comprise in excess of 80 % of the solvent extractable lipids from seawater, but little is known of their composition other than that the mixtures are complex, which makes calibration uncertain [9]. Pigments and degradation products are often in high abundance, and it is rarely possible to identify and quantify individual phospholipids or glycolipids [9, 12], unless the sample is dominated by relatively undegraded organic matter such as occurs during phytoplankton blooms.

3.2.2 Application to Environmental Studies

Measurement of Hydrocarbons

Hydrocarbon distributions in environmental samples are usually very complex, so their analysis and quantitation is generally performed by capillary gas chromatography (GC) with FID and/or mass spectrometric detection [28]. These techniques enable identification and quantification of individual compounds characteristic of petroleum contamination. It is, however, our experience that the total concentration of hydrocarbons in polluted samples is often difficult to quantify by GC since only a few percent of the hydrocarbons are present as identifiable, discrete peaks. This is particularly

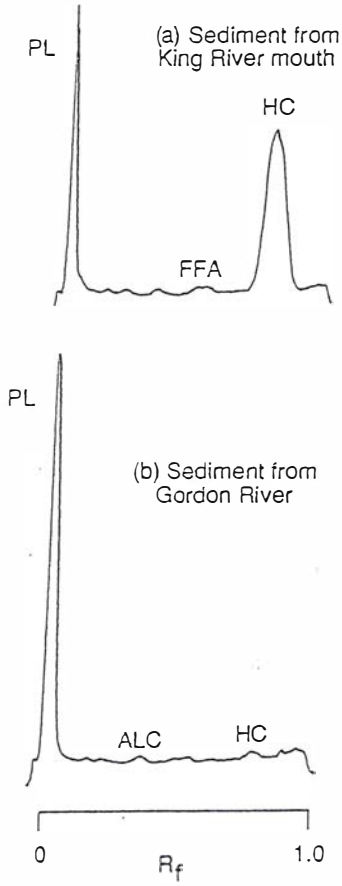

Figure 1

Iatroscan TLC-FID chromatograms of sedimentary lipids from two sites in Macquarie Harbour, Tasmania (Australia). (a) mouth of King River, (b) Gordon River. Solvent system: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Refer to Table 1 for peak identifications.

Table 1

Abbreviations used for lipid classes in figures and text

Lipid class	Abbreviation	Lipid class	Abbreviation
Hydrocarbon	HC	4-Methyl sterol	4-MS
Squalene	SQ	Alcohol	ALC
Wax ester	WE	5β-Stanol	5β-ST
Sterol ester	SE	4-Desmethyl sterol	ST
Diacylglycerol ether	DAGE	Dihydroxy sterol	DHS
Triacylglycerol	TG	Diacylglycerol	DG
Ketone	KET	Polar lipid	PL
Free fatty acid	FFA	Unidentified	UN

so when large amounts of unresolved complex material (UCM) from biodegraded petroleum residues are present. In such cases, TLC-FID provides a quick and direct measure of total hydrocarbons in complex environmental mixtures.

An example of the use of TLC-FID for measuring hydrocarbons in estuarine sediments collected on the west coast of Tasmania (Australia) is illustrated in **Figure 1.** The two samples, when analyzed by capillary GC, both contained a similar abundance and distribution of long chain *n*-alkanes. Sediment from the polluted King River site, however, contained a higher proportion of short chain alkanes with low carbon preference index together with a large amount of UCM. The TLC-FID analysis clearly reveals the high level of petroleum-derived hydrocarbons in the King River sediment; the concentration of hydrocarbons in the two samples determined by TLC-FID was 70 μg g^{-1} (King River) and 6 μg g^{-1} (Gordon River).

A further example of the use of TLC-FID in hydrocarbon assays is for the quantitation of algal-derived β-carotene which cannot be measured by capillary GC. β-Carotene is a major hydrocarbon in many algae and it is a significant constituent of hydrocarbon fractions isolated from sediments rich in algal-derived organic matter. The mismatch in hydrocarbon concentrations determined by TLC-FID and GC-FID in such samples can often arise from the presence of β-carotene.

TLC-FID is a very useful technique for determining abundances of aliphatic and aromatic hydrocarbons in tar samples or heavy crude oils. Concentrations of these hydrocarbon classes in light oils and condensates can, however, be underestimated due to losses of the more volatile liquid *n*-alkanes and smaller aromatic molecules such as alkylbenzenes and naphthalenes. This appears to be a result of volatilization of the compounds from the rod before they reach the collector due to the heat from the FID flame. Losses from the rods during solvent development can also be significant.

Detection of Sewage-Derived Organic Matter in Marine Samples

A recent application of the TLC-FID technique in our laboratory has been the analysis of organic constituents of sewage and the determination of their fate in the marine environment. TLC-FID has proven particularly useful for quantitating the "grease" components of sewage which include hydrocarbons, triacylglycerols, free fatty acids, sterols, and polar lipids. The typical TLC-FID chromatogram shown in **Figure 2** illustrates the high concentrations of free fatty acids that are commonly found in sewage.

There has been considerable recent interest in the use of coprostanol (5β-cholestanol) as a tracer of sewage in the marine environment [29]. 5β-Stanols are separated from 4-desmethyl sterols under the conditions used to separate the sewage mixture (Figure 2), so TLC-FID can be used as a quick screening technique for environmental samples. The 5β-stanol peak includes coprostanol and any 24-ethyl-5β-cholestanol that might be present. The latter component can

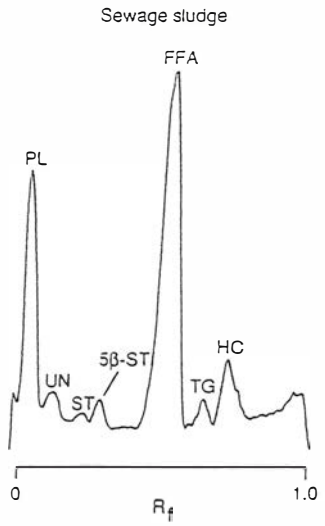

Figure 2

Iatroscan TLC-FID chromatogram of sewage sludge lipids. Solvent system: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Refer to Table 1 for peak identifications.

be difficult to separate from 24-methylcholesterol using GC techniques, so TLC-FID offers an alternate method to quantify total 5β-stanols in field samples. Where terrestrially-derived long-chain alcohols or algal-derived 4-methyl sterols are present in a sample, care needs to be taken since these are not separated from 5β-stanols by many solvent systems.

3.2.3 Applications to Mariculture and Nutrition

The nutritional importance of lipids and essential polyunsaturated fatty acids (EFA) in the diets provided to fish and shellfish, particularly at the larval stages, in mariculture operations is now well recognized [30 – 32]. TLC-FID can readily be used to gain further knowledge on the nutritional requirements and quality of commercial species and intermediate feed organisms such as rotifers and brine shrimps. As rotifers generally synthesize only small amounts of ω3 EFA, the EFA must be provided to the rotifers in their food to meet the possible demand of the commercial species. For example, rotifers are usually reared on yeast and subsequently given a diet rich in EFA (either using algae or a dietary supplement) before being given to fish or other marine animals.

In our laboratory, we have applied TLC-FID in rotifer nutrition studies to quantify the lipid composition of rotifers under different feeding regimes [33]. Polar lipids predominate in yeast-fed rotifers. Following feeding with the prymnesiophyte alga *Pavlova lutheri,* which is commonly used in mariculture in Australia [34], the triacylglycerol content of the rotifers increased steadily (**Figure 3**). The triacylglycerol fatty acid composition of oyster spat reflects the animal's diet, whereas the composition of fatty acids in the polar lipids is less dietary dependent [32]. It is likely that a higher triacylglycerol content in rotifers may be nutritionally beneficial to marine animals fed on them.

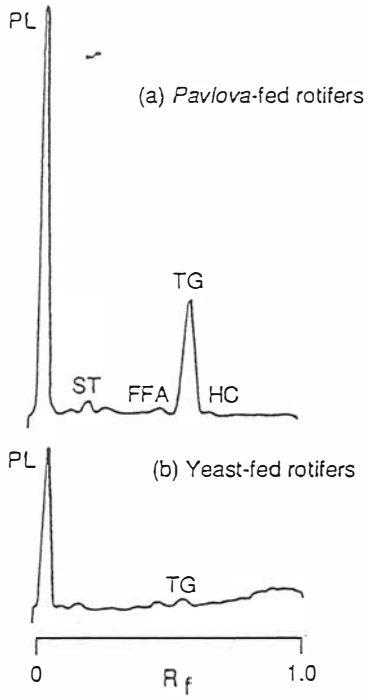

Figure 3

Iatroscan TLC-FID chromatograms of rotifer lipids. (a) rotifers fed on the microalga *Pavlova lutheri*, (b) rotifers fed on yeast. Solvent system: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Refer to Table 1 for peak identifications.

3.2.4 *Analysis of Marine Oils*

The use and production of marine-derived oils is increasing in Australia and overseas. In many cases, further utilization of fish by-catch and by-products has the potential to add considerable value to fisheries which are already at maximum catch levels. TLC-FID can provide a rapid and quantitative analysis of the lipid composition of these fish oils and value-added products. Representative chromatograms of a range of marine oils are presented in **Figure 4 a – d.**

Fish Oils

The growth of the mariculture industry both in Australia and overseas has increased the need for high quality oils for feeding both to the commercial species and to intermediate organisms such as brine shrimp, rotifers and copepods. The requirement for a high content of EFA is well recognized by the mariculture industry, but the quality of oils available varies considerably. TLC-FID analysis of a variety of feedstocks shows that there can be significant differences between oils in the proportion of triacylglycerol, free fatty acid and polar lipid (*e.g.* Figure 4 a, b); this affects the nutritional value of the product.

Orange roughy oil differs from most fish oils in that it consists almost exclusively of wax ester (Figure 4 c). Oils rich in wax esters have uses in the pharmaceutical, tanning and steel-manufacturing industries [35]. The proportion of free fatty acids is often a key parameter in determining oil quality; TLC-FID analysis enables not only direct quantitation of the wax ester content, but also the determination of the free fatty acid content.

Squalene from Shark Liver Oils

The C_{30} isoprenoid hydrocarbon squalene has many uses in the pharmaceutical and chemical industries as a lubricant and cosmetic base. The livers of deep water sharks can con-

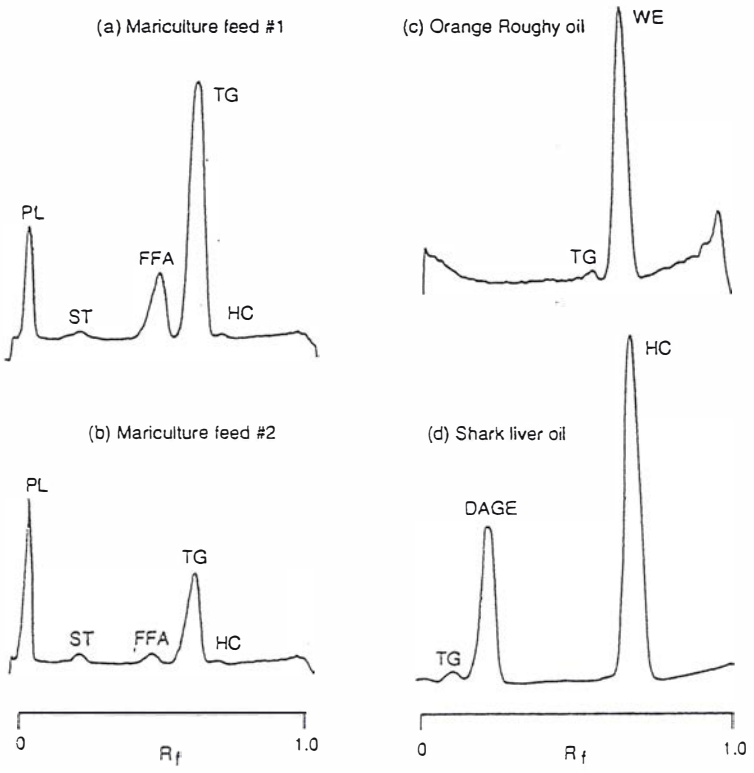

Figure 4

Iatroscan TLC-FID chromatograms of marine oils. (a) mariculture feed # 1, (b) mariculture feed # 2, (c) orange roughy oil, (d) shark liver oil. Solvent system for samples a, b, and c: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Solvent system for sample d: hexane-diethyl esther (96 + 4; *v/v*). Refer to Table 1 for peak identifications

tain up to 70 % by weight of squalene and thus have excellent potential as natural sources of this compound. We recently conducted a survey of the lipid composition of livers from sharks caught to the south of Australia [36]. TLC-FID provided a rapid screening of the oil for squalene content as well as quantitative data for the other major neutral lipid constituents, triacylglycerols (TG) and diacylglyceryl ethers (DAGE) (Figure 4 d). The hexane-diethyl ether (96 + 4, *v/v*) solvent system provided excellent separations of the 3 major lipid classes. The lipid compositions of the shark liver oils were found to vary significantly between species. Wax esters were generally very minor components, even though orange roughy was a significant part of the diet of some species. Capillary GC analysis confirmed that squalene comprised over 99 % of the total hydrocarbons. The amounts of pristane were very small, and so the concentration of total hydrocarbons determined by TLC-FID provided a good measure of the squalene content in the oils.

Fats and Oils from Microalgae

Recognition that certain species of marine algae can contain substantial quantities of lipid when grown under suitable conditions, has prompted investigations of their potential as sources of ω3 polyunsaturated fatty acids for use by the pharmaceutical and health industries [37]. TLC-FID provides a convenient monitoring tool to assess how the oil content of microalgae varies when grown under different environmental conditions and permits rapid optimization of oil yield. A major advantage of the use of the TLC-FID system in this case is that only 200 ml or less of algal culture needs to be grown to provide sufficient material for analysis, thus providing significant savings in time and chemical usage. This becomes very important in multifactorial experiments in which nutrient, light and temperature are varied in order to modify the biochemical composition of the microalgae for the production of specific chemicals or to obtain a high lipid content.

Over the past few years we have undertaken a survey of the lipid abundances in species from the major classes of microalgae grown in laboratory culture [38, 39] which has made considerable use of TLC-FID. Polar lipids usually predominate in cultures grown under favourable nutrient and light levels and harvested during exponential growth. The abundances of sterols, free fatty acids and triacylglycerols are also readily measured, but very few species have been found that contain significant concentrations of hydrocarbons or wax esters.

The TLC-FID system is also very useful as a screening technique for identifying species that contain unusual lipids. Figure 5 shows TLC-FID chromatograms for the lipid extracts of the marine diatom *Chaetoceros gracilis* and the prymnesiophyte alga *Pavlova lutheri*. The chromatogram for *C. gracilis* is typical of the lipids found in many microalgae, although the relative abundance of free fatty acids is somewhat higher than in most algae. This contrasts with the chromatogram obtained for *P. lutheri* which shows several additional peaks. Subsequent isolation of the compounds, followed by GC and GC-MS analysis showed that this alga contains unusual 4-methyl sterols (peak labeled 4-MS) and a previously unknown class of dihydroxylated sterols (peak labeled DHS) which was the most abundant neutral lipid present [40]. Note that with this solvent system *n*- alcohols coelute with 4-methyl sterols. Pigments or diacylglycerols (DG) which give rise to a small peak in the same region as the dihydroxylated sterols (as in *C. gracilis*) can also be present in the extract. In such

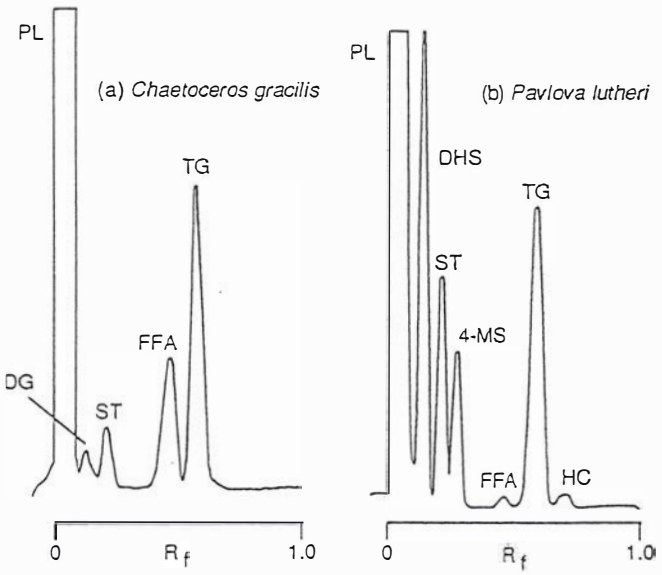

Figure 5

Iatroscan TLC-FID chromatograms of lipids of marine algae. (a) *Chaetoceros gracilis* (diatom), (b) *Pavlova lutheri* (prymnesiophyte). Solvent system: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Refer to Table 1 for peak identifications. In *C. gracilis*, DG also contained unidentified pigment material.

cases, one would then need to use more polar solvent systems to confirm that unusual lipids are indeed present: 1,3-diglycerides, 1,2-diglycerides and sterols are, for example, completely resolved by hexane-diethyl ether-acetic acid 60 + 40 + 0.2 (*v/v*).

TLC-FID is also a very useful technique for monitoring changes in the lipid composition of samples of phytoplankton in the field. An example of the analysis of the lipid composition of a field population of sea-ice diatoms collected from McMurdo Sound, Antarctica over a 6 week period is shown in Figure 6. Significant changes in lipid composition were observed which could be related to changes in the physiology of the algal community during the bloom period [41]. These changes mimic similar responses found in microalgae cultured in the laboratory.

4 Conclusion

TLC-FID has secured a place in the analytical laboratory as an adjunct to more traditional analytical systems such as gas chromatography and high performance liquid chromatography. It has unique advantages and its own special features. In little more than a decade it has advanced from prototypes to a well developed commercially available system. The in-

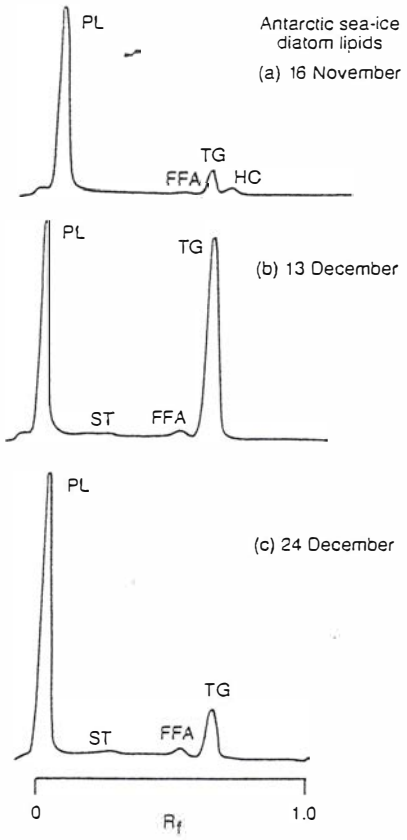

Figure 6

Iatroscan TLC-FID chromatograms of lipids from field populations of sea-ice diatoms collected in McMurdo Sound, Antarctica during the austral summer. Collection dates as shown. Solvent system: hexane-diethyl ether-acetic acid (60 + 17 + 0.2; *v/v*). Refer to Table 1 for peak identifications.

troduction of better sample application systems, detector improvements and advances in data handling combined with new detectors such as the flame thermionic ionization detector (FTID) [42, 43] will ensure the continued use of this technique.

Other techniques will undoubtedly rise to compete with TLC-FID for some applications. TLC followed by scanning densitometric detection of derivatized lipids may offer a more sensitive alternative to Iatroscan TLC-FID, particularly if fluorescent derivatives can be employed. Continued progress in the development of a universal, non-destructive HPLC detector for lipids [17] may also provide alternative analytical strategies.

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Recent Developments with Marine Oil Products in Australia

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The Australian Science and Technology Council (ASTEC), in a 1988 report on the fishing industry, stated "fish are big business in this country, but we could get an even better return from our catch". Most of the by-product from finfish is processed to low value pet food, fishing bait, fish meal or unrefined fish oils. The potential exists for production and export of higher value-added products from fish oil and meal. ASTEC indicated that better use of the by-catch (non-targeted species) was also essential if we are to avoid wasting our limited fishery resource. The Marine Products Project of the CSIRO Division of Oceanography has developed a considerable intellectual resource on marine oils and has also assembled state of the art facilities for the chemical characterization of these oils. Research undertaken by the group draws together the combined expertise of industry and CSIRO with the objective of exploiting the by-catch and by-products of Australia's south-eastern fisheries, in particular orange roughy, jack mackerel, blue grenadier, deep-sea shark and other species.

Oil products with potential for development include: (i) oils rich in wax esters from orange roughy and other species, (ii) squalene and other components from the livers of deep-sea sharks and (iii) oils enriched in omega-3 polyunsaturated fatty acids (PUFA) from Australian species. Recent progress on the characterization of such oils and the manufacture of value-added products from the oils is reported here.

Oil Analyses

Sample extraction: Oils were extracted quantitatively by the modified one-phase $CHCl_3$-MeOH Bligh and Dyer method[1] and stored under nitrogen at -20 °C until analysis.

Lipid composition was determined with an Iatroscan MK III TH10 TLC-FID analyzer (Iatron Laboratories, Japan)[2] on silica gel SIII chromarods (5 μm particle size) developed in (i) hexane/diethyl ether/acetic acid (60/17/0.5; v/v/v) or (ii) hexane/diethyl ether (96/4; v/v). Solvent (i) resolved hydrocarbons, triacylglycerols and free fatty acids as well as other common neutral lipid classes while polar lipids remained at the origin. Solvent (ii) (but not (i)) separated hydrocarbons from wax esters and diacylglyceryl ethers from triacylglycerols. After development, the chromarods were oven-dried for 10 min at 100 °C and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class (0.1-5 μg range). Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, WA) on IBM-compatible PCs.

Sample fractionation: Sterol and fatty acid fractions were saponified in 5% KOH in MeOH/water, 80/20, v/v (80 °C, 3 hr). Sterols and other non-saponifiable lipids were extracted into hexane/$CHCl_3$ (4/1, v/v) and converted to trimethylsilyl ethers (TMSi ethers) by treatment with BSTFA (60 °C, 60 min). The remaining aqueous layer was acidified, the fatty acids extracted with hexane/$CHCl_3$ (4/1, v/v) and were converted to fatty acid methyl esters (FAME) by treatment with methanol/$CHCl_3$/HCl (10/1/1; v/v/v; 100 °C, 60 min). The same procedure was also used for direct transesterification of fatty acids from the total extract. Products were extracted into hexane/$CHCl_3$ (4/1; v/v) and stored at -20 °C.

Gas chromatography analyses: Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890 GC equipped with a 50 m x 0.32 mm i.d. cross-linked methyl silicone (HP1) fused-silica capillary column and a flame ionization detector.[1,3] Methylnonadecanoate or methyltricosanoate were used as the internal injection standard. Hydrogen was used as the carrier gas. Selected fatty acid samples were also analyzed using a 50 m x 0.25 mm i.d. polar BP20 capillary column. Shark liver oils were additionally analyzed with a 3 m x 0.25 mm i.d. BPX5 fused-silica capillary column (SGE).[4] Peak areas were quantified as before. Identification of individual components was based on comparison of retention time data with those obtained for authentic and laboratory standards.

GC-mass spectrometry analyses (GC-MS): GC-MS analyses of selected samples were performed on a HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and an on-column injector. Data were acquired and processed on an HP 59970C Workstation operated in scan aquisition mode.[1,3] The nonpolar column was similar to that described above.

Nomenclature: Fatty acids are designated as number of carbon atoms: number of double bonds. The number (x) of carbon atoms of the closest double bond from the methyl end (n) of the molecule is given by (n-x). For example, eicosapentaenoic acid (EPA) is designated 20:5(n-3). All subsequent double bonds in PUFA are methylene interrupted. The prefixes i and a indicate iso and anteiso branching, and the suffixes c and t indicate *cis* and *trans* geometry respectively.

Wax Ester Rich Oils

The discovery of the first orange roughy (*Hoplostethus atlanticus*) spawning aggregation in Australian waters in 1989 resulted in the largest commercial catches taken to date for this species.[5] Combined catches from northeastern and southern Tasmania in 1989-90 exceeded 34,000 tonnes; just under a half of the catch came from the spawning aggregation off St. Helens.[6] A further 16,000 tonnes was taken from the spawning aggregation in winter 1990. A spawning aggregation, from which 800 tonnes was catch, was located in the Great Australian Bight

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John K. Volkman (MRACI) is a Senior Principal Research Scientist with the CSIRO Division of Oceanography and leader of the Marine Resources and Pollution Program. Prior to joining CSIRO in 1983, he undertook postdoctoral studies at Bristol University (Organic Geochemistry Unit) and Woods Hole Oceanographic Institution. He has 20 years experience in the fields of marine lipids, organic geochemistry and petroleum geochemistry and has published over 85 papers. His work has included studies of polyunsaturated fatty acids in fish and microalgae, squalene in sharks and lipids in sediments and seawater.

Table 1: Lipid class composition of commercially produced orange roughy oil and waste oil produced during composting of orange roughy waste with eucalypt waste.

Lipid class	Percentage composition	
	Orange roughy oil	Waste oil
Wax ester	97.1	92.4
Triacylglycerol	1.9	0.9
Free fatty acid	Not detected	4.7
Fatty alcohol	Not detected	1.6
Polar lipid	1	0.4

in July 1990.[7] Of the orange roughy caught, approximately 30% is processed into fillets.[8] The remainder of the fish until recently was largely discarded as waste. Methods of handling and processing orange roughy oil and fish meal have been reviewed.[8] Prior to the late eighties, however, limited work had been undertaken within Australia on further processing of orange roughy waste. This was due in part to a general lack of knowledge of the composition of the waste and its possible uses. A major aim of our research has been to provide high quality biochemical data on orange roughy and the waste obtained from processing. We expect that this knowledge will be readily transferable to the utilization of other fish and crustacean by-products and by-catches.

Orange roughy waste contains one major and one minor component that have substantial market value: oil and pigment. Approximately 18% of the whole fish consists of oil.[9] About 80% of this oil is in waste products — the head, swim bladder, frame and skin. Over 2,500 tonnes of crude oil were discarded with the waste material of the 1989 catch alone. In 1989, this oil was worth $2.5 m based on an estimate of $1 per kg; the refined oil is worth considerably more. Orange roughy oil, unlike the oil from most other commercial fish, is composed almost entirely of wax esters (Table 1, see also[10,11]). To date much of the oil produced in Australia has been exported to Japan for use as a lubricant for steel manufacturing. Compared with conventional triacylglycerol-rich fish oils the high stability of the wax ester oil at elevated temperature makes it ideally suited for such uses. Orange roughy oil is a possible substitute for jojoba oil which has been used as a replacement for sperm whale oil.[9] These oils are used in the pharmaceutical and cosmetic industries after being refined, decolorized and deodorized. At present, the oil is refined in Japan and sells for $25 per kg. This is an area for future development and there is ample scope to produce the value-added high-quality oil in Australia.

Our work has shown that the oil present in orange roughy caught in Australian, New Zealand and British waters has a similar composition.[10,11] The major fatty acids in the commercially-produced oil in decreasing order of abundance are: 18:1(n-9)c, 20:1(n-9)c, 16:1(n-7)c, 18:1(n-7)c and 22:1(n-11)c. The major alcohols: 20:1(n-9)c, 16:0, 22:1(n-11)c and 18:1(n-9)c (Figure 1). The oil is rich in monounsaturated constituents and, compared with most fish oils, it contains very low levels of polyunsaturated fatty acids (PUFA). These results have been passed on to local processors who previously

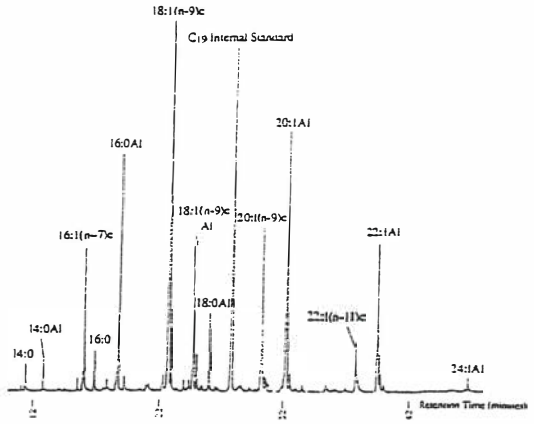

Figure 1: Partial gas chromatogram showing fatty acid and fatty alcohol composition of orange roughy oil. HP1 capillary column. The suffix AI denotes alcohol.

imported oil from New Zealand. Further work is required to determine the composition and physical properties of oil derived from the variety of processes that will be used by industry.

Anecdotal reports by fishermen are that wet weather gear rotted, and loose paint and greases were removed from metal surfaces by orange roughy oil. Beku Environmental Products Ltd. working with CSIRO has developed biodegradable cleaning products using marine oils which are now marketed. The products were trialled on the Research Vessel *Aurora Australis* and at the Australian Antarctic bases Mawson and Davis during the summer of 1991/92 (Figure 2). The degreaser and hand-cleaner products compete with petrochemical or organochlorine based products which are clearly less biodegradable and considerably more toxic than marine oil based products. As an additional spin-off, our work has also highlighted possible variations in the minor components of the oil with different sized fish and different catch sites. These results may prove useful in defining discrete stocks of orange roughy.

The second component of potential value in the waste is the pigment. Wild salmon have flesh with a characteristic redness, however farmed salmon require the addition of pigment (carotenoids) to the feed. As a local source of the red pigment is not available, commercially

Figure 2: Field trials in Antarctica of a degreaser product made from marine oil.

Table 2: Major fatty acid composition of selected fish and algae

Fatty acid	Percentage composition						
	Fish					Micro Algae	
	Spiney icefish	Patagonian toothfish	Jack mackerel	Field zooplankton	Salmon farm waste	Diatoms (n=4)	Prymnesiophytes (n=4)
14:0	5.2	2.8	7.3	2.4	4.2	15.2	17.1
16:0	15.8	16.2	15.7	15.7	15.7	15.4	15.3
18:0	2.4	3	3.1	3.3	4.2	1.6	0.4
16:1(n-7)c	7.5	5.4	5.1	3.9	5.1	27.5	9.7
18:1(n-9)c	8.9	17.7	9.9	1.9	16.5	2.1	0.7
18:1(n-7)c	7	5.9	2.9	3.9	3.5	0.5	0.4
20:1(n-9)c	0.8	8.3	8.3	0.3	3.3	< 0.1%	< 0.1%
18:2(n-6)	1.9	1.1	1.7	0.9	6.6	0.7	1.7
20:5(n-3)	20.6	8.4	10.9	22.2	7.1	10.3	24.1
22:6(n-3)	24.5	20.3	11.5	29.2	15.7	1.8	9.7
Others	5.4	10.9	23.6	16.3	18.1	24.9	20.9

produced synthetic pigment is added to the food used by the Australian aquaculture industry. The current product is a high cost synthetic and a natural source may also be a more attractive alternative. The orange coloration from orange roughy or other species may provide a source of carotenoids for the feeds used for salmon and other farmed animals. Work is underway to characterize these pigments and develop methods for their isolation.

A third use of the waste is as a plant growth medium. Tas Crays produce a soil additive by composting of orange roughy waste with eucalypt waste. The product was initially sold in Tasmania as "OR-90" and more recently has been released in the mainland states as "Fish and Chips". New Zealand industry had previously composted orange roughy with pine waste. This is more desirable than dumping the waste which had caused environmental problems in Tasmania during 1989. The waste oil that drains off during the composting procedure has (Table 1) a high wax ester content as wax esters are considerably more stable than conventional (triacylglycerol-containing) fish oils. After appropriate treatment, this oil will also be suitable for industrial use.

Even if the 1989 and 1990 catch levels of orange roughy are not sustained, all indications are that a major deepwater fishery is likely to continue in Australia. Hence there will continue to be a resource of material for possible oil, pigment, meal and fertilizer production. By enhancing post-harvest production of orange roughy and converting waste material into value-added by-products, the economic yields of the fishery can be maximized at whatever level is achieved.

Other wax ester rich oils are also being pursued. For example, oreo dories are caught commercially in the same areas as orange roughy, and based on initial analyses, the oil from dories is also rich in wax esters. The fatty acid and alcohol distribution differs from orange roughy, however it may be possible to substitute or at least blend the oil with orange roughy oil.

Omega-3 Oils

Polyunsaturated fatty acids reduce the incidence of coronary heart disease and stroke in humans.[12,13] This has considerably enhanced the image of fish as a healthy food amongst consumers. Capsules of fish oils containing high levels of the essential polyunsaturated fatty acids, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], are aggressively marketed overseas, and have captured a small market in Australia (e.g. Maxepa and related products). More recently (n-3) oils have been incorporated in other food items; for example, in Scandinavia the oils are now added to bread. The next generation of (n-3) oils contain considerably higher levels of EPA and DHA (approaching 70-80%) and processing of the raw oils is required to achieve these high levels.

The demand for fish oils by the mariculture industry must also continue to increase. The economics of fish farming rely on the supply of inexpensive feeds of a suitable quantity and quality. Tasmania has fortunately had large catches of jack mackerel, which contains 25-30% EPA plus DHA (Table 2). When this fishery failed in 1989, oils had to be imported at considerable cost. At that time, data was not available on possible local replacements. By building the data base on the oil composition of Australian species, we will be much better placed to identify alternative feedstocks and to take advantage of new market opportunities.

A range of oils from Australian species have now been analyzed and results indicate that they show potential for use in mariculture feed and in other industries. For example, the southern ocean species, Patagonian toothfish (*Dissostichus eleginoides*) and spiny icefish (*Chaenodraco wilsoni*), both contain appreciable levels of EPA and DHA (Table 2). Large amounts of these species were processed in Hobart during the 1991/1992 summer and, based on lipid compositional data, the oil from these species may be suitable for use by the mariculture industry. Other oils analyzed which contain high levels of EPA and DHA are (i) oil produced from waste from the salmonid industry, (ii) oil from certain species of zooplankton grown in sewage-treatment ponds (Table 2), and (iii) red bait (*Emmelichthys nitidus*) which is a by-catch associated with the jack mackerel fishery.

Similar technologies can be exploited to isolate valuable oils from algal and bacterial sources. Currently a number of groups in Australia are growing the green alga *Dunaliella* for the commercial production of natural β-carotene. The development of appropriate technology and facilities for the production of value-added marine oils will be readily transferred to production of fine chemicals from microalgae and possibly bacteria. Several microalgal species produce high levels of (n-3, range 5-20%) polyunsaturated fatty acids and others produce arachadonic acid [20:4(n-6)], which is highly sought after by the pharmaceutical industry. For example diatoms produce EPA (mean = 11%) with only low levels of DHA present, and certain prymnesiophyte species produce large amounts of both EPA (24%) and DHA (10%) (Table 2). Such algae are being used in mariculture operations as sources of these fatty acids which are considered essential for the growth and survival of the larval stages of many animal species. We also recently have examined the fatty acid composition of Antarctic bacteria. It was generally thought that bacteria do not produce EPA and DHA. The knowledge that other organisms produce higher quantities of EPA and DHA at low temperatures led us to examine new strains of bacteria isolated from Antarctic waters. Early results indicate several strains do produce EPA, albeit at low levels. In time chemicals made by microalgae, such as essential fatty acids, could be obtained and purified using technology developed for the production of value-added products from fish oils.

Shark Liver Oil

Deep sea sharks represent a significant, but very under-utilized by-catch of the orange roughy and other fisheries. When orange roughy is not abundant, up to 50% of the catch may be sharks.[14] Livers make up about 20% of the shark's weight and contain considerable quantities of oil often enriched in the C_{30} unsaturated isoprenoid hydrocarbon squalene. Squalene has been used in Asia as a health food or can be hydrogenated to squalane for use in the pharmaceutical and cosmetic industries as a lubricant and cosmetic base. At present, small quantities of unprocessed shark liver oil are sold to Japan for up to $5/kg. There is scope to increase exports or refine the oil to value-added purified products. Squalene accounts for up to 80% of shark liver oil in some commercial Australian oils (Figure 3). The CSIRO Division of Oceanography and Division of Chemicals and Polymers have developed a process to isolate squalene at > 99% purity from the raw oil.

Shark liver oils also contain significant amounts of diacylglyceryl ethers. The liver of one sample of *Centroscymnus squamosus* contained 79% diacylglyceryl ethers (DAGE), but contents around 20-30% are more common in laboratory scale processing of individual species[15] and 10-50% has been found in commercial production. DAGE have

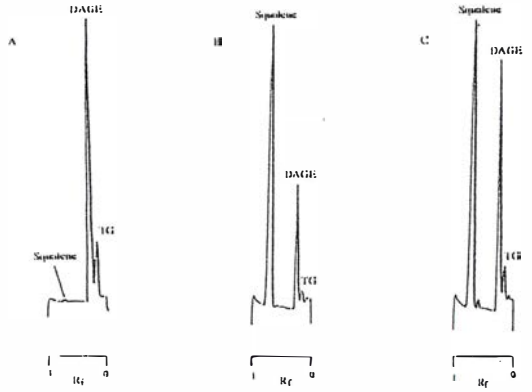

Figure 3: *Iatroscan TLC-FID chromatograms of three commercially-produced shark liver oils. Solvent: hexane/diethyl ether (96/4, v/v). Abbreviations: DAGE, diacylglycerol ether; TG, triacylglycerol.*

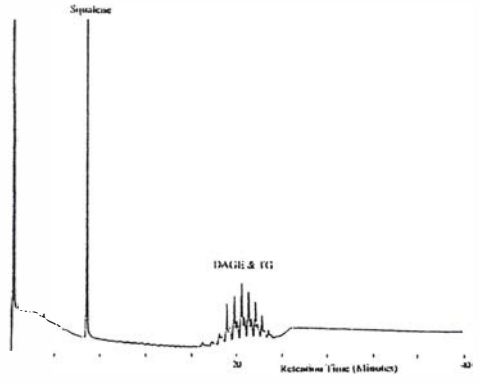

Figure 4: *High temperature gas chromatogram of shark liver oil (BPX5 capillary column). Temperature ramp: 50-400 °C. Abbreviations: DAGE, diacylglyceryl ether; TG, triacylglycerol.*

structures similar to triacylglycerols, with one of the esters replaced by an ether which makes them much more stable. Specialist techniques based on TLC-FID have now been developed which allow us to quantify these compounds in any type of marine oil (Figure 3). More recently we have been able to use high temperature (400 °C) capillary gas chromatography to analyze shark liver oil directly (Figure 4). Several imported health products containing DAGE derived from deep-sea sharks are available in Australia. The products are said to be high in alkylglycerols which exist in our body in the bone marrow, liver, spleen and in mothers milk. Again the potential exists in the first round to at least replace an imported product with local goods. If the opportunity arises, an export market could then be targeted. Full exploitation of this resource will depend on a better understanding of which species contain marketable quantities of squalene and diacylglyceryl ethers. A preliminary survey[14,15] was restricted to 8 shark species commonly caught in waters to the west and east of Tasmania. The data base needs to be expanded to include other species, including those caught in other Australian waters.

Conclusions

Orange roughy oil and shark liver oils, together with oils derived from other deepwater species, have specialist applications because of their unusual compositions. Most marine oils, however, consist mainly of triacylglycerols which are rich in polyunsaturated fatty acids. The markets for marine oils continue to expand, but we know far too little about the oil content and composition of most Australian fish. We have conducted limited studies on species marketed in Tasmania,[12] but these have been restricted to fish for human consumption rather than analyses of the by-catch (trash fish) or of waste products. Both areas offer scope for future commercial developments.

In summary, value-added marine oil products can be manufactured from both the by-catch of established fisheries and from waste generated during seafood processing. The products have uses in the aquaculture, pharmaceutical, food and other industries. Increasing government and public concern is being directed at minimizing waste generated by industry, including aquaculture and seafood processing. This provides a further stimulus for the production of value-added marine oil products from these wastes.

Acknowledgement

This work was supported in part by funding from FRDC and ASAC. Nick Elliott and Stephanie Davenport, CSIRO Fisheries, Hobart and Chris Strauss and Greg Simpson, CSIRO Chemicals and Polymers, Clayton are thanked for many valuable discussions and ongoing support. The officers and crew of *Aurora Australis* and fellow ANARE personnel are thanked for their assistance during field trials of marine oil products. Our colleagues in the Marine Resources and Pollution Program are thanked for assistance with various aspects of the project. BEKU Environmental Products, Scales, Trident Seafoods, Tas Crays, the Australian Antarctic Division, Allison Laboratories, Seafish Tasmania, Deer Horn, Zootech and others are thanked for provision of materials and their interest and support of the project.

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INFORM
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Developments with Marine Oil Products in Australia

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In recent years a number of companies have established facilities for the production of oil and high grade meal from the south-east Australian fisheries. The Marine Products Project of the CSIRO Division of Oceanography has developed a considerable intellectual resource on Australian marine oils and has assembled state of the art facilities for the chemical characterisation of these oils. Research undertaken by the group draws together the combined expertise of industry and CSIRO with the objective of exploiting the by-catch and by-products of Australia's south-eastern fisheries, in particular the larger, and economically important catches of orange roughy, jack mackerel and blue grenadier and other species including deep-sea shark.

Oil products which have been or have the potential for development in Australia include: (i) oils rich in wax esters from orange roughy and other species, (ii) squalene and other components from the livers of deep-sea sharks and (iii) oils enriched in omega-3 polyunsaturated fatty acids (PUFA) from Australian species. Commercial products have been previously developed overseas from these three types of oils. The lipid composition of fish can depend, however, on seasonal changes in feeding habits, changes in available food, regional differences in the basic foods and nutrients, and other factors. Progress on the characterisation of oils derived from commercially-landed Australian species and related samples together with the manufacture of value-added products from these oils are reported.

Oil analyses

Sample extraction

Oils were either supplied directly by industry or were obtained by extraction of fish and related using the modified one-phase $CHCl_3$-MeOH-H_2O Bligh and Dyer method. Antarctic bacterial isolates were obtained courtesy of the Australian Collection of Antarctic Microorganisms (ACAM), Hobart, Tasmania. After phase separation, the lipids were recovered in the lower $CHCl_3$ layer (solvents were removed in vacuo) and were made up to a known volume and stored sealed under nitrogen at -20°C until analysis.

Lipid composition

The abundance of major lipid classes present in the oils was determined using an Iatroscan MK III TH10 TLC-FID analyser (Iatron Laboratories, Japan) [1]. Samples were applied to silica gel SIII chromarods (5 µm particle size), which were developed in either (i) hexane/diethyl ether/acetic acid (60/17/0.2; v/v/v) or (ii) hexane/diethyl ether (96/4; v/v). Solvent mixture (i) resolves hydrocarbons, triacylglycerols and free fatty acids as well as other neutral lipid classes. Polar lipids remained at the origin and were not separated. Solvent mix (ii) was used to separate hydrocarbons from wax esters and diacylglyceryl ethers from triacylglycerols. These classes are not separated with solvent mix (i). After development, the chromarods were dried for 10 min at 100°C and analysed. The flame ionization detector (FID) was calibrated for each compound class (0.1-10 µg range). Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, Western Australia) operated using an IBM-compatible personal computer.

Sample fractionation

Sterol and fatty acid fractions were obtained following alkaline saponification of an aliquot of the total lipids (3 ml, 5% KOH in MeOH/water, 80/20, v/v; 80 °C, 3 hr). After the addition of water, non-saponifiable lipids including sterols were extracted into hexane/$CHCl_3$ (4/1, v/v) and converted to their corresponding trimethylsilyl ethers (TMSi ethers) by treatment with N,O-bis(trimethylsilyl)-trifluoroacetamide (50 µl, 60°C, 60 min). The remaining aqueous layer was acidified and the fatty acids extracted with hexane/$CHCl_3$ (4/1, v/v). The liberated fatty acids were converted to fatty acid methyl esters by treatment with 2 ml methanol/$CHCl_3$/HCl (10/1/1; v/v/v; 100°C, 60 min). The same methylation procedure was also used for direct transesterification of fatty acids from the total extract. Products were extracted into hexane/$CHCl_3$ (4/1, v/v) and stored at -20°C.

Gas chromatography analyses

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890 GC equipped with a 50 m x 0.32 mm i.d. cross-linked methyl silicone (HP1) fused-silica capillary column and an FID [2]. Where used, either methylnonadecanoate or methyltricosanoate was the internal injection standard. Hydrogen was used as the carrier gas. Selected fatty acid

samples were analysed using a 50 m x 0.32 mm i.d. polar BP20 capillary column. Shark liver oils were also analysed directly using a 3 m x 0.32 mm i.d. BPX5 fused silica capillary column (SGE Australia). For all GC analyses, peak areas were quantified using DAPA chromatography software as previously described. Identification of individual components was based on comparison of retention time data with that obtained for authentic and laboratory standards.

GC-mass spectrometry analyses (GC-MS)

GC-MS analyses of selected samples were performed on a HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and either an on-column or JADE valve injector (SGE Australia). Data were acquired and processed on an HP 59970C Workstation operated in scan aquisition mode[2]. The nonpolar column was similar to that described above.

Nomenclature

Fatty acids are designated as number of carbon atoms: number of double bonds. The number (x) of carbon atoms of the closest double bond from the methyl end (n) of the molecule is given by (n-x). For example, eicosapentaenoic acid (EPA) is designated 20:5(n-3). All subsequent double bonds in PUFA are methylene interrupted. The prefixes i and a indicate iso and anteiso branching, and the suffixes c and t indicate cis and trans geometry respectively.

Wax ester rich oils

The discovery of the first orange roughy (*Hoplostethus atlanticus*) spawning aggregation in Australian waters in 1989 resulted in the largest commercial catches taken to date for this species. Combined catches from Tasmanian waters in 1989-90 exceeded 34,000 tonnes. Nearly half the catch came from the spawning aggregation off St. Helens (northeast Tasmania). A further 16,000 tonnes were taken from the spawning aggregation in winter 1990. A smaller spawning aggregation, from which 800 tonnes were caught, was located in the Great Australian Bight in July 1990. Of the orange roughy catch, approximately 30% is processed into fillets [3]. Until recently the remainder of the fish was largely discarded as waste.

Methods of handling and processing orange roughy oil and fish meal have been reviewed [3]. Prior to the late 1980s, however, limited work had been undertaken within Australia on further processing of waste derived from orange roughy. In part, this was due to a general lack of information on the composition of the waste and its possible uses. A major objective of our work has been to provide high quality biochemical data on orange roughy and the waste obtained from processing, thereby assisting Australian industry to better utilize the orange roughy resource. The knowledge derived will be transferable to the utilisation of other fish and crustacean by-products and by-catches.

Orange roughy waste contains one major and one minor component that have substantial market value: oil and pigment. The fish contains copious quantities of oil; approximately 18% of the whole fish consists of oil [4]. About 80% of this oil is located in the waste products - the head, swim bladder, frame and skin. Over 2,500 tonnes of crude oil were discarded with the waste material of the Australian 1989 catch alone. In 1989, this oil was worth $2.5 m based on an estimate of Aust $1 per kg; the refined oil is worth considerably more.

Orange roughy oil, unlike the oil from most other commercial fish, is composed almost exclusively of wax esters (Table 1, see also [5,6]). Much of the oil produced in Australia to date has been exported to Japan for use as a lubricant in the steel manufacturing industry. Compared with conventional triacylglycerol-rich fish oils, the high stability of the wax ester oil at elevated temperature makes it ideally suited for use by industry. It is the same stability of the wax ester-rich oil (compared to triacylglycerol oils) that caused early reports of adverse gastrointestinal effects after eating orange roughy. It was subsequently demonstrated that normal consumption of orange roughy by man does not lead to dietary problems as a result of consuming wax esters.

The composition of orange roughy oil makes it a possible substitute for jojoba oil which has been used as a replacement for sperm whale oil [4]. These oils are utilised by the cosmetic and pharmaceutical industries after being refined, decolorized and deodorized. At present, the oil is refined in Japan and sells for Aust $25 per kg. This is an area for future development and there is scope to produce the value-added high-quality oil in Australia.

Previous work has shown that the oil present in orange roughy caught in Australian, New Zealand and British waters has a similar distribution of chemical components [5]. The oil is rich in monounsaturated constituents and, compared with most fish oils, it contains very low levels of polyunsaturated fatty acids (PUFA). The major fatty acids in the commercially-produced oil in decreasing order of abundance are: 18:1(n-9)c, 20:1(n-9)c, 16:1(n-7)c, 18:1(n-7)c and 22:1(n-11)c. The major alcohols are: 20:1(n-9)c, 16:0, 22:1(n-11)c and 18:1(n-9)c (Table 2). These results were good news for Australian companies previously importing oil from New Zealand, in that there was a local source available. These results have been passed on to local processors. Ongoing work is required to determine the composition and physical properties of oil derived from the variety of processes that will be used by industry.

The composition of wax ester-rich oils derived from other commercially-landed species is also being pursued. For example, oreo dories are caught in the same areas as orange roughy, and based on initial analyses, the oil from several of the dories can also be rich in wax ester (Table 1); this oil, like orange roughy oil, is rich in monounsaturated components (Figure 1). The fatty acid and alcohol distribution differs slightly from that found for orange roughy, however, it may be possible to substitute or at least blend the oil with that produced from orange roughy.

Anecdotal reports by fisherman provided evidence of the solvating properties of orange roughy oil. Wet weather gear was rotted, and loose paint and greases were removed from metal surfaces by the oil. Beku Environmental Products Ltd working in association with CSIRO has developed biodegradable cleaning products using marine oils which are now being marketed. The products were successfully trialled at the Australian Antarctic bases Mawson and Davis during the summer of 1991/92 (Figure 2). The degreaser and hand-cleaner products compete with currently used petrochemical or organochlorine-based products. The latter are considered to be less biodegradable and more toxic than the marine oil-based products.

Another use of waste from the orange roughy fishery is in plant growth products. New Zealand industry had previously composted orange roughy with pine waste. In Australia a similar process is used, only eucalypt waste replaces the pine waste. The orange roughy waste is mixed with plant material and applied to soil in horticultural practices. Such processing of the oil and other material is more desirable than dumping the waste which had caused

environmental problems in Tasmania during 1989. The waste oil that drains off during the composting procedure has also been analysed (Table 1). The high wax ester content of the oil is maintained during composting as wax esters are considerably more stable than conventional (triacylglycerol-containing) fish oils. After appropriate treatment, this oil may also be suitable for use by industry.

Even if the 1989 and 1990 catch levels of orange roughy are not sustained, all indications are that a major deepwater fishery is likely to continue in Australia. Hence there will continue to be a resource of material for possible oil, pigment, meal and fertilizer production. By enhancing post-harvest production of orange roughy and converting waste material into value-added by-products; the economic yields of the fishery can be maximized at whatever the level of fishing that can be sustained.

Shark liver oil

Deep sea sharks represent a significant, but very under-utilized by-catch of the orange roughy and other deep-water fisheries. When orange roughy is not abundant, up to 50% of the catch may be sharks [7]. The livers of these sharks are very large (about 20% of the total shark's weight), and they contain considerable quantities of oil which is often enriched in the $C_{30}H_{52}$ unsaturated isoprenoid hydrocarbon squalene. Squalene has been used in Asian countries as a health-food and lotion or can be converted by hydrogenation to squalane which has a variety of uses in the pharmaceutical and cosmetic industries, particularly as a lubricant and cosmetic base oil. At present, shark liver oil is sold unprocessed into the Japanese market. There is scope to increase the amounts exported and to further refine the oil into value-added purified products.

Squalene accounts for up to 80% of shark liver oil in some commercially produced Australian oils (Figure 3). The CSIRO Division of Oceanography and Division of Chemicals and Polymers have jointly developed a process to isolate squalene at greater than 99% purity from the raw oil. The opportunity now exists for Australian industry to export the value-added refined squalene, rather than the crude shark liver oil.

Shark liver oils can also contain significant amounts of diacylglyceryl ethers (Figure 3). DAGE

have chemical structures similar to triacylglycerols, but one of the ester bonds is replaced by an ether bond which makes the compounds much more stable. The liver of one sample of *Centroscymnus squamosus* contained 79% diacylglyceryl ethers (DAGE), but contents of around 20-30% are found in laboratory analyses of individual species. Shark liver oil from commercial production has been found to contain between 10-50% DAGE. Australian industry presently imports these compounds from overseas, so there is potential for import replacement and possibly for the development of an export market. Techniques based on TLC-FID have been developed[1], which allow us to quantify these compounds in any type of marine oil (Figure 3). More recently we also have been able to use high temperature (400 °C) capillary gas chromatography to analyse shark liver oil directly.

The DAGE-rich oils also may have commercial uses. Several imported health products containing DAGE derived from deep-sea sharks are available in Australia. The products are said to be high in alkylglycerols which exist in the bone marrow, liver, spleen and in mothers milk. Again the potential exists to replace an imported product with Australian goods. If the opportunity arises, a wider export market could then be targeted.

Full exploitation of the deep-sea shark resource will depend on a better understanding of which species contain marketable quantities of squalene and diacylglyceryl ethers. A preliminary survey [7] was restricted to 8 species commonly caught in waters to the west and east of Tasmania. The data base needs to be expanded to include other species, including those caught in other Australian waters.

Omega-3 oils

It is generally believed that polyunsaturated fatty acids reduce the incidence of coronary heart disease and stroke in humans. This finding has enhanced the image of fish as a healthy food for consumers. Capsules of fish oils containing high levels of the essential polyunsaturated fatty acids, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], are marketed aggressively internationally, and have captured a small market in Australia (e.g. Maxepa and related products). More recently (n-3) oils have been incorporated in other food items; for example, the oils are now added to bread in Scandanavia. The next generation of (n-3) oils contain considerably higher levels of EPA and DHA (approaching 70-80%) and

processing of the raw oils is required to achieve these high levels.

The demand for fish oils by the mariculture industry (both in Australia and overseas) will also continue to increase. The economics of fish farming relies on the supply of inexpensive feeds of a suitable quantity and quality. Tasmania fortunately has had large catches of jack mackerel, the oil of which contains 25-30% EPA plus DHA (Table 2). These two fatty acids are considered to be essential for most species reared by the mariculture industry. When the jack mackerel fishery failed in 1989, oils had to be imported into Australia at considerable cost. At that time, data was not available on possible local replacements. By building the data base on the oil composition of Australian species, we will be much better placed to identify alternative feedstocks and to take advantage of new market opportunities.

A range of flesh samples and oils from Australian fish species and related samples have been analysed and results indicate that they do show potential for use in mariculture feed and in other industries. For example, flesh from the oreo dories, the southern ocean species, Patagonian toothfish (*Dissostichus eleginoides*, data not shown) and spiny icefish (*Chaenodraco wilsoni*), contain appreciable levels of EPA and DHA (Table 2). Large amounts of the southerm ocean species were processed in Hobart during the 1991/1992 summer and, based on lipid compositional data, the oil from these species may be suitable for use by industry. Other oils analysed which contain high levels of EPA and DHA are (i) oil produced from waste from the salmonid industry, (ii) oil from certain species of zooplankton grown in sewage-treatment ponds and (iii) red bait (*Emmelichthys nitidus*) which is a by-catch associated with the jack mackerel fishery (Table 2).

Similar technologies can be exploited to isolate valuable oils from algal and bacterial sources. Currently a number of groups in Australia are growing the green alga *Dunaliella* for the commercial production of natural β-carotene. The development of appropriate technology and facilities for the production of value-added marine oils may be transferred to production of fine chemicals from microalgae and possibly bacteria. Several microalgal species produce high levels of (n-3) polyunsaturated fatty acids (range 5-20%) and others produce arachidonic acid [20:4(n-6)]. For example diatoms produce EPA with only low levels of DHA present, and certain prymnesiophtye species produce large amounts of both EPA and DHA. Such algae are being used in mariculture operations as sources of these fatty acids which are considered

essential for the growth and survival of the larval stages of many animal species.

We recently have examined the fatty acid composition of Antarctic bacteria. Although a limited number of bacteria have been shown to produce PUFA, it has been generally thought that most bacteria do not produce EPA and DHA. The knowledge that other organisms produce higher quantities of EPA and DHA at low temperature lead us to examine new strains of bacteria isolated from Antarctic waters. Early results indicate that the proportion of Antarctic strains that produce EPA is considerably higher than found for temperate marine bacteria; we have found EPA levels up to nearly 20% of the total fatty acids. Similarly, a number of strains that produce DHA (up to 3%) have also been isolated. Representative fatty acid compositional results for two strains of Antarctic bacteria are shown in Table 2. In addition to the presence of EPA (6.3% of total fatty acids) in strain NS831, the level of monounsaturates was considerably higher than observed for many fish species; the level of saturated fatty acids was lower than in fish. In time, chemicals made by microalgae and bacteria, such as essential fatty acids, could be obtained and purified using technology developed for the production of value-added products from fish oils.

Orange roughy oil and shark liver oils, together with oils derived from other deepwater species, have specialist applications because of their unusual compositions. Most marine oils, however, consist mainly of triacylglycerols which are rich in polyunsaturated fatty acids. The markets for marine oils continue to expand, but we know far too little about the oil content and composition of most Australian fish. Limited studies have been conducted on species marketed in Tasmania [8], but these have been restricted to fish for human consumption rather than analyses of the by-catch (trash fish) or of waste products. Both areas offer scope for future commercial developments.

In summary, value-added marine oil products can be manufactured from both the by-catch of established fisheries and from waste generated during sea-food processing. The products have uses in the aquaculture, pharmaceutical, food and other industries. Increasing government and public concern is being directed at minimizing waste generated by industry, including from aquaculture and seafood processing. This provides a further stimulus to Australian industry for the production of value-added marine oil products from these wastes.

Acknowledgement

This work was supported in part by funding from FRDC and ASAC. Nick Elliott and Stephanie Davenport, CSIRO Fisheries, Hobart, Chris Strauss and Greg Simpson, CSIRO Chemicals and Polymer, Clayton and Graeme Dunstan and John Volkman, CSIRO Oceanography, Hobart are thanked for provision of samples, valuable discussions and ongoing support. The officers and crew of *Aurora Australis* and fellow ANARE personnel are thanked for their assistance during field trials of marine oil products. Our colleagues in the Marine Resources and Pollution Program are thanked for assistance with various aspects of the project. BEKU Environmental Products, Scales, Trident Seafoods, Tas Crays, Squalus, Sydney-Wide Seafoods, the Australian Antarctic Division, Allison Laboratories, Seafish Tasmania, Deer Horn, Zootech and others are thanked for provision of materials and their interest and support of the project.

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Figure legend

Figure 1. Partial gas chromatogram showing fatty acid and fatty alcohol composition of dory oil. HP1 capillary column. The suffix Alc denotes alcohol.

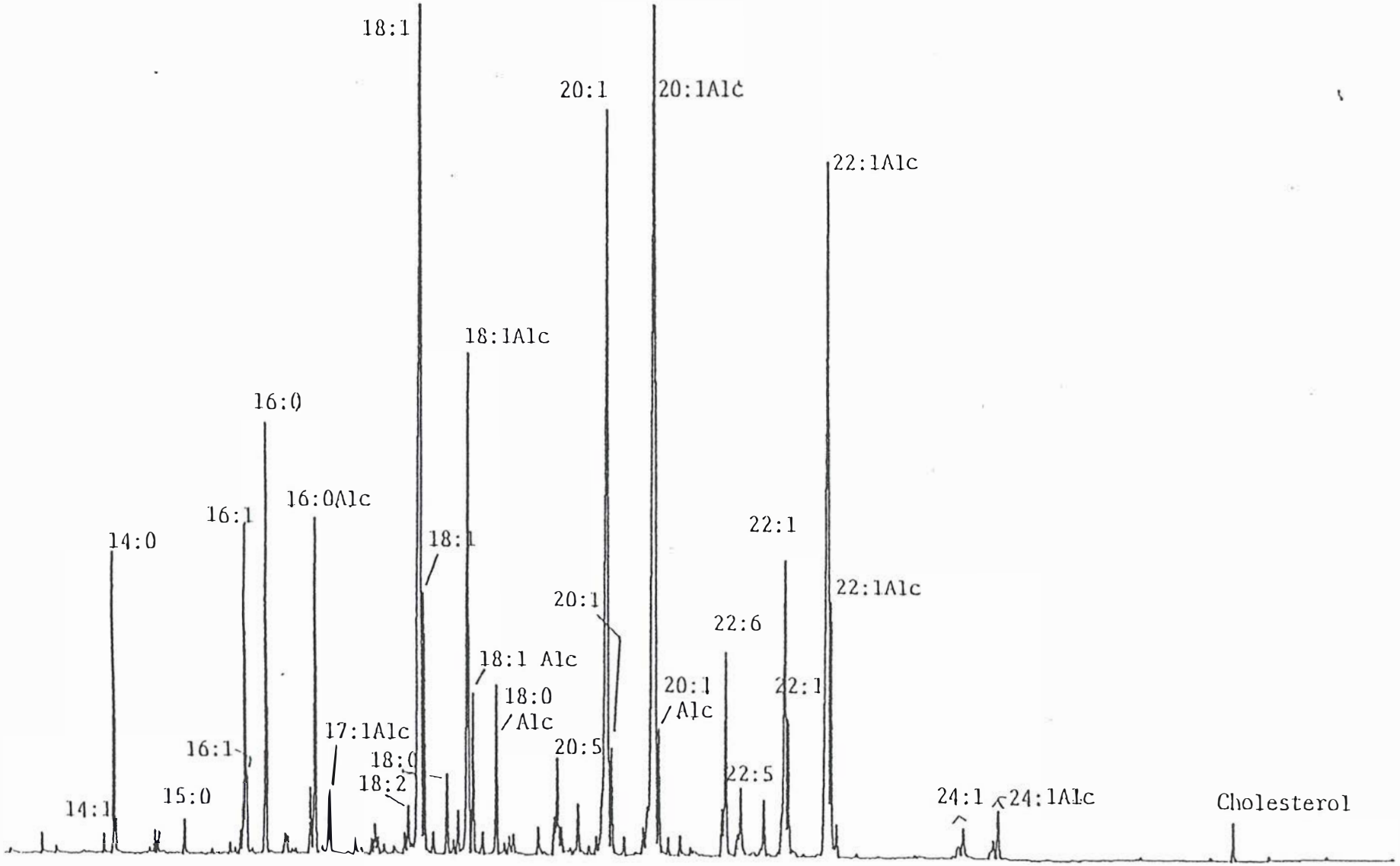
Figure 2. Field trials in Antarctica of a degreaser product made from marine oil.

Figure 3. Iatroscan TLC-FID chromatograms of two commercially-produced shark liver oils. Solvent: hexane/diethyl ether (96/4, v/v). Abbreviations: HYC, hydrocarbon (predominately squalene); DAGE, diacylglyceryl ether; TG, triacylglycerol; FFA, free fatty acid.

Tables

Table 1. Lipid class composition of commercially produced orange roughy and dory oils and waste oil produced during composting of orange roughy waste with eucalypt waste.

Table 2. Major fatty acid composition of selected fish and bacteria.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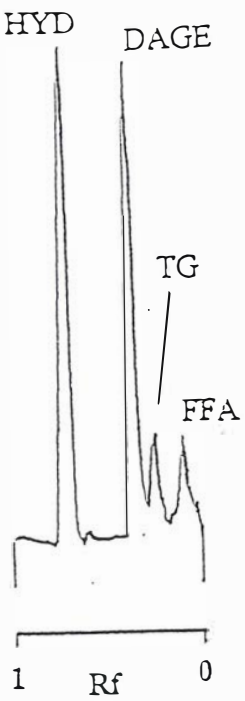
Cholesterol

Fig 1

A



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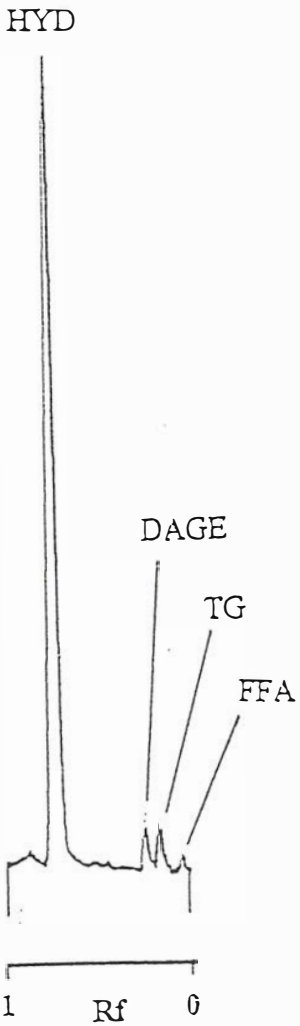

Table 1: Lipid class composition of commercially produced orange roughy and dory oils and waste oil produced during composting of orange roughy waste with eucalypt waste.

Lipid Class	Percentage composition		
	Orange roughy oil	Waste orange roughy oil	Dory oil
Wax ester	97.1	92.8	90.9
Triacylglycerol	1.9	-	9.1
Free fatty acid	Not detected	4.1	-
Fatty alcohol	Not detected	2	-
Sterols	-	-	tr
Polar lipid	1	1.1	tr

tr: trace, <0.5%

Table 2: Major fatty acid composition of selected fish and bacteria

Fatty acid	Percentage composition								
	Fish							Bacteria	
	Spikey dory	Black dory	Spiney icefish	Jack Mackerel	Lagoon zooplankton	Salmon farm waste oil	Orange roughy oil	Strain NSB31	Strain JS6P2W
14:0	1.4	2.7	5.2	7.3	1	4.2	1.7	0.5	7.5
16:0	35	20.4	15.8	15.7	11.8	15.7	3.3	7.5	23.3
18:0	8.6	3.8	2.4	3.1	4.5	4.2	0.7	tr	0.5
16:1(n-7)c	2.4	3.6	7.5	5.1	2.7	5.1	10.7	48.4	36.9
18:1(n-9)c	22.4	20.9	8.9	9.9	10.5	16.5	50.6	0.8	0.2
18:1(n-7)c	2.8	3.7	7	2.9	5.8	3.5	5.1	5.6	1.4
20:1(n-9)c	4	13.7	0.8	8.3	0.6	3.3	17.9	-	-
22:1(n-11)c	-	-	-	-	-	-	4.1	-	-
24:1	-	-	-	-	-	-	1.2	-	-
18:2(n-6)	0.5	1	1.9	1.7	10.5	6.6	-	tr	-
20:5(n-3)	3.7	5.9	20.6	10.9	17.2	7.1	-	6.3	-
22:6(n-3)	12.7	13.4	24.5	11.5	0.8	15.7	0.1	-	2.3
Others	6.5	10.9	5.4	23.6	34.6	18.1	4.6	30.9	27.9

Comp. Biochem. Physiol. (Submitted)

LIPID, FATTY ACID AND SQUALENE COMPOSITION OF LIVER OIL FROM SIX SPECIES OF DEEP-SEA SHARKS COLLECTED IN SOUTHERN AUSTRALIAN WATERS

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Abstract-1. The liver oils from the deep-sea sharks *Somniosus pacificus*, *Centroscymnus plunketi*, *Centroscymnus crepidater*, *Etmopterus granulosus*, *Deania calcea* and *Centrophorus scalpratus* were analysed to describe their lipid, fatty acid and squalene compositions.

2. The major lipids in all species of shark were diacylglyceryl ethers and triacylglycerols and the major hydrocarbon was squalene. Only trace levels of polar lipids were detected.

3. Monounsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{20:1}$, $C_{22:1}$ and $C_{24:1}$) comprised 62-84% of the fatty acids. Saturated fatty acids contributed 11-26% of the total fatty acids while polyunsaturated fatty acids were relatively minor components (1-13%).

4. All sharks had different lipid compositions, but similar fatty acid and diol profiles.

5. The high squalene content (50-82% of oil) of all species except *Centroscymnus plunketi* and *Somniosus pacificus* suggests that the oil from these deep-sea sharks collected in southern Australian waters will be suitable for industrial uses.

INTRODUCTION

The proportion of sharks caught in the southern Australian deep-sea fishing industry during fishing trawls can be significant (average research trawl contains 26% shark; can be up to 50% or greater). The amount of shark landed varies due to factors such as seasonal variation (migration etc.) and reproductive status of fishes (Davenport and Deprez, 1989). In the past when sharks were only a minor bycatch, they were either discarded or used as fishmeal (King and Clark, 1987). Apart from fillets, some of the current products directly resulting from shark include skin, fins (soup), fish meal, corneas, cartilage, chondroitine, liver oils and a large number of indirect products including surface active agents, aromatics, lubricants, artificial silk, cosmetics, rubbers and pharmaceuticals (Buranudeen and Richards-Rajadurai, 1986; Gopakumar and Thankappan, 1986).

An international market for shark liver oil has existed for some time. The literature to date has suggested that it would be difficult to sustain a commercial shark liver oil industry in Australia and New Zealand due to such factors as their low reproductive rate and slow growth (e.g. King and Clark, 1987). More efficient utilisation of shark waste should therefore be considered.

The oil derived from deep-sea shark livers usually contains high levels of squalene, diacylglyceryl ethers and triacylglycerols and minor levels of free fatty acid, sterol, pristane, wax ester and sterol ester (Deprez *et al.*, 1990). The lipids found as major components, diacylglyceryl ethers and triacylglycerols, and the hydrocarbon squalene, are believed to assist in the function of the liver as a hydrostatic organ (Craik 1978; Deprez *et al.* 1990; Malins and Barone 1970; Sargent *et al.* 1973).

Although a number of Australian operations have been exporting crude shark liver oil for several years, the value of the purified oils and lipids available from the processing of the shark

bycatch has not been fully realised. This resource has been recognised by many countries and as a result, purification and marketing techniques for shark liver oil products have been established (King and Clark, 1987; Gopakumar and Thankappan, 1986).

The oil composition of livers from several species of deep-sea shark landed in south-east Australia has been reported (Davenport and Deprez, 1989; Deprez *et al.*, 1990). In this study, further results are reported for Australian species, several of which form significant commercial resources and for which no published data are available (e.g. *Somniosus pacificus, Etmopterus granulosus* and *Centroscymnus plunketi*). The shark liver oil samples were predominantly obtained from commercial processes rather than by solvent extraction of whole livers as used in some previous studies. A comparison between the lipid and fatty acid compositions of these southern Australian sharks will provide further insight into the abundance of specific lipids in the liver of deep-sea sharks. These results could assist industry with the exploitation of the liver from these sharks, material currently regarded by some as waste.

MATERIALS AND METHOD

Samples and Lipid Extraction

Somniosus pacificus (Pacific sleeper shark) was caught south of Maatsuyker Island (southern Tasmania, Australia) on 29 January 1993 at approximately 1000 m depth. The shark was an immature female, 429 cm in total length. A section of the liver was removed and stored at -20°C until analysis. Shark liver oil samples from *Centroscymnus plunketi* (plunket shark), *Centroscymnus crepidater* (deep-sea dogfish), *Etmopterus granulosus* (lantern shark), *Deania calcea* (platypus shark) and *Centrophorus scalpratus* (Endeavour dogfish) were supplied by local fishermen. The processed oils were obtained by maceration of the livers, followed by separation and decantation of the oil; conditions were as used by industry for commercial production of shark liver oil. For *D. calcea* and *C. scalpratus*, the number of livers was not determined. The oils for the remaining species were composite samples derived from between 1 and 6 livers. The catch location and water depth for each species were not recorded.

A portion of the liver from *Somniosus pacificus* was homogenised using a mortar and pestle and a known mass was 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 (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.

Lipids

An aliquot of the total solvent extract (TSE) or the diluted crude liver oil was analysed using an Iatroscan MK III TH10 TLC-FID analyser to determine the abundances of individual lipid classes (Volkman and Nichols, 1991). Samples were applied in 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), a mobile phase resolving non-polar compounds such as wax esters, triacylglycerols and free fatty acids. A second non-polar solvent system of hexane-diethyl ether (96:4 v/v) was also used to resolve hydrocarbon, triacylglycerol and diacylglyceryl ethers. After development, the chromarods were oven dried and analysed immediately to minimise adsorption of atmospheric contaminants. The flame ionisation detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, triolein; 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 and Nichols, 1991).

Fatty Acids, Glyceryl Ether Diols and Squalene

An aliquot of the TSE was treated with potassium hydroxide in methanol (5% w/v) and saponified under nitrogen for 3 hours at 80°C. Neutral lipids (e.g. glyceryl ether diols and hydrocarbons) were then extracted into hexane/chloroform (4:1 v/v, 3x1.5 mL) and transferred to sample vials. Following acidification of the supernatant aqueous layer using hydrochloric acid (pH=2), fatty acids were methylated to their corresponding fatty acid methyl esters (FAME) using methanol-hydrochloric acid-chloroform (10:1:1 v/v/v; 80°C, 2 hrs). Products were then extracted into hexane/chloroform (4:1 v/v, 3x1.5 mL) and stored at -20°C. The neutral lipid fractions were treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide

(BSTFA 50 μL, 80°C, 1 hr) to convert sterols to their corresponding TMS (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 and a split/splitless injector. 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 minute, the oven temperature was raised to 150°C at 30°C/min, then to 250°C at 2°C/min and finally to 300°C at 3°C/min. Peaks were quantified with software from DAPA Scientific Software, Kalamunda. Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. All GC results are subject to an error of ±5%.

Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a HP 5890 GC and a 5970 Mass Selective Detector (MSD) fitted with a direct capillary inlet and JADE-valve injector (SGE, Australia). Data were acquired on a HP 59970C Workstation operated in scan acquisition mode. The GC was fitted with a capillary column similar to that described above.

Determination of Double Bond Configuration in Fatty Acids and Glyceryl Ether Diols

Dimethyl disulphide (DMDS) adducts of monounsaturated fatty acids and glyceryl ether diols were formed by treating the total fatty acid and glyceryl ether diol 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 TMS derivatives prior to GC-MS analysis.

RESULTS AND DISCUSSION

Lipid Composition

The lipid composition of the oil extracted from livers of six species of deep-sea sharks found in southern Australian waters was determined. The major lipids included diacylglyceryl ethers (DAGE), triacylglycerols (TAG) and free fatty acids (Table 1). Hydrocarbon, predominantly the hexa-unsaturated isoprenoid squalene ($C_{30}H_{50}$), was also a major component in all of the sharks analysed (50-81% of total lipid), except *Somniosus pacificus* and *Centroscymnus plunketi* (0 and 1% respectively). DAGE and TAG were present in all shark species at levels between 10-77% and 5-49% respectively. Only *Etmopterus granulosus (2)* contained free fatty acids (8%), possibly resulting from degradation of polar lipid or TAG and DAGE prior to analysis. The absence of free diols was suggestive of negligible DAGE degradation, although little is known on the behaviour of glyceryl ether diols produced during processing. Polar lipids were either present in low abundance (<2%) or were not detected in the processed oils from the southern Australian sharks analysed.

The distribution of lipids in the liver oil from *Centrophorus scalpratus* was dominated by hydrocarbon (predominantly squalene, 82%) with minor levels of DAGE (10%) and TAG (9%). *Deania calcea* and *Centroscymnus crepidater* liver oils also contained high levels of squalene (69 and 73% respectively) and this is in good agreement with previous reports for these two species (Deprez *et al.*, 1990; Sargent *et al.*, 1973). The high levels of squalene are typical of liver oils from deep-water sharks inhabiting water depths between 600 and 1000 metres (Buranudeen and Richards-Rajadurai, 1986; Deprez *et al.*, 1990).

Centroscymnus plunketi (plunket shark) liver oil was comprised of predominantly DAGE (77%) and TAG (23%). The trace amount of squalene present in *C. plunketi* liver oil is similar

to the level determined by Summers (1987) for a sample of this species collected in New Zealand waters.

The composition of liver oils from *Somniosus pacificus* and *Etmopterus granulosus* has not been previously documented. The liver oil of *S. pacificus* was found to contain high levels of both DAGE (50%) and TAG (49%) whereas the liver oil from *E. granulosus (1)* (commonly caught in deep-sea fishery trawls in southern Tasmanian waters), comprised squalene (60%) and DAGE (30%) with lower levels of TAG and polar lipids (7 and 1% respectively). Liver oil from a second sample of *E. granulosus (2)* contained a slightly different lipid profile - lower levels of squalene (50%) and higher levels of DAGE (32%), TAG (9%) and free fatty acids (8%). The difference in lipid composition between the two samples, particularly the level of squalene (Fig. 1), may be due to differences in processing time (i.e. during liver processing, the first oil layer released contains higher levels of squalene) (Richard Saul, personal communication).

The role of specific lipids and hydrocarbons as buoyancy regulators in the liver of deep-sea sharks has been documented (Malins and Barone, 1970; Craik, 1978) and it is apparent that different sharks regulate liver lipid composition to maintain buoyancy. The levels of squalene, DAGE and TAG are also affected by the dietary intake of the specific components and seasonal factors (Hayashi and Takagi, 1981; Kayama, 1971). The absence of more precise literature on the role of these three lipid classes, along with physical variables such as depth of catch, geographical location and sex of specimens analysed prevents further interpretation of the reasons for the large differences observed in the liver lipid compositions. Furthermore, examination of previous literature data (Batista and Nunes, 1992; Deprez *et al.*, 1990; Kayama *et al.*, 1971; Sargent *et al.*, 1973) together with the results of this study, show that there are large within genus variations (*Centroscymnus*, *Centrophorous* and *Etmopterus*) in levels of hydrocarbon (squalene), DAGE and TAG. Further studies which examine the effect of depth and geographical distribution on liver oil composition are warranted.

Squalene and Other Hydrocarbons

Of the six different species analysed, the liver oil of four species contained greater than 50% squalene (i.e. >500 mg/g oil on a quantitative basis). Squalene concentrations were in the range of 500 and 820 mg/g oil in all species except *Centroscymnus plunketi* and *Somniosus pacificus* (Fig. 1); the former contained no squalene and the latter contained 9 mg squalene/g oil. Only four of the six species examined had high levels of squalene, suggesting that the relationship between high levels of squalene and living in deep waters reported by Buranudeen and Richards-Rajadurai (1986) may not hold for all species. This study along with previous reports, has indicated that some deep-sea sharks contain both high levels of DAGE and squalene (e.g. *E. granulosus*) or DAGE without squalene (*C. plunketi*). This feature is believed to account for the lower levels of squalene in some species.

The amount of squalene found in the liver from *C. crepidater* analysed in this study was higher than found by Deprez *et al.* (1990) (73% versus 61%). On the other-hand, the level of squalene in *C. scalpratus* and *D. calcea* (82% and 70%), is in good agreement or greater than values determined in other studies where levels were in the order of 75% (e.g. Gopakumar and Thankappan, 1986; Deprez *et al.*, 1990). The difference observed in the two analyses of *C. crepidater* may be due to a variety of factors; seasonal variation and within species, differences in oil composition has previously been documented (Summers, 1987). Alternately, differences in processing conditions may account for the variation observed. The higher squalene value obtained in the present study was for commercially produced oil, whereas the value measured by Deprez *et al.* (1991) was for whole livers treated by solvent extraction. The latter technique may not be representative of normal processing conditions.

The oil from the liver of *Etmopterus granulosus* contained approximately 50-60% squalene. This species can be taken in Tasmanian waters in large quantities and it is believed that this

level will be high enough to support isolation of the liver and extraction of the oil from this species on a commercial scale.

Pristane, a hydrocarbon commonly found in shark oils, was found at trace levels in all of the species analysed in this study. Pristane is of dietary origin with zooplankton generally being the predominant source. Based on its origin, pristane levels in shark liver oils would be expected to vary depending on dietary and seasonal factors, and within and between shark species. Although pristane and squalene have similar physical characteristics and chemical properties, the difference in the length of the carbon skeleton results in a significant difference in their physical properties. A distillation step may be required to remove the trace quantities of pristane present in shark liver oils if necessary.

Fatty Acid Content and Composition

The fatty acid composition and total fatty acid content of the six species of shark analysed is presented in Table 2. The predominant fatty acids in all species were the monounsaturates (62-84%), 18:1(n-9)c, 20:1(n-9)c and 22:1(n-11)c. Minor levels of 24:1(n-9)c, 22:1(n-7)c and 16:1(n-7)c were also detected. *Centroscymnus plunketi* liver oil comprising mainly DAGE and TAG, had the highest levels of monounsaturated fatty acids (84%) while *Centrophorus scalpratus*, which contained squalene as the dominant lipid, contained the lowest level of monounsaturated fatty acids (62%).

Saturated fatty acids were present at between 11 and 26% of the total fatty acids while polyunsaturated fatty acids were only minor components (1-13%). The major saturated fatty acid was 16:0 (9-21%) while other saturates including 14:0, 15:0, 17:0, 18:0, 20:0 and 22:0 were present at much lower levels. Polyunsaturated fatty acids (PUFAs) accounted for between 1 and 13% (mean=6.3%) of the total fatty acids. PUFA detected included 22:6(n-3), 18:2(n-6), 20:5(n-3), 20:4(n-6), 18:3(n-3), 22:5(n-3), 22:5(n-6) and 22:4(n-6).

Docosahexaenoic acid (DHA, 22:6(n-3)) was the most abundant of the PUFAs comprising up to 7% of the total fatty acids in *S. pacificus*. The role of 20:5(n-3) and 22:6(n-3) in lipid fluidity has been previously documented (e.g. Cowey and Sargent, 1977; Russell, 1990) and the high levels of DHA in *S. pacificus* may complement the high levels of DAGE and TAG in this species and play a role in maintaining their fluidity.

Total fatty acid content was between 95 and 575 mg/g (Table 2) with *C. plunketi* containing the highest amount of fatty acid and *C. scalpratus* the lowest. The sharks that contained the highest levels of squalene had the lowest total fatty acid contents. Total fatty acid content may also vary with catch depth, as observed for squalene based on the relationship between catch depth and squalene content reported by Buranudeen and Richards-Rajadurai (1986).

Glyceryl Ether Diols (derived from DAGE)

The Iatroscan (TLC-FID) analyser provides a rapid means of detecting DAGE without any sample treatment and derivatisation prior to analysis. The process of saponification used in this study converted the DAGE to the corresponding 1-alkyl glyceryl ether diols. The diols are then extracted with other non-saponifiable neutral lipid material and converted to di-O-trimethylsilyl (TMS) ether derivatives prior to analysis by gas chromatography. GC/MS analysis of the samples readily identified the diol components from their base peak at m/z=205.

Diacylglyceryl ethers were found at varying levels in the seven samples analysed in this study (10 - 77%). The major diols (as a percentage of the total diols) were: 1-hexadecylglyceryl ether (16:0; 14-18%), hexadec-7-enylglyceryl ether (16:1(n-7)c; 3-11%), octadecylglyceryl ether (18:0; 3-17%), octadec-7-enylglyceryl ether (18:1(n-7)c; 5-6%), octadec-9-enylglyceryl ether (18:1(n-9)c; 42-62%) and eicosa-9-enylglyceryl ether (20:1(n-9)c; 1-12%) (Fig. 2). A similar profile was observed for all shark liver oils, and this distribution of alkyl chains is generally similar to the fatty acid profiles with the exception of lower levels of PUFA and

$C_{22:1}$. For example, the diol profile is dominated by monounsaturated side-chains (16:1(n-7)c, 18:1(n-9)c, 18:1(n-7)c and 20:1(n-9)c), with only minor levels of saturated diols (14:0, 16:0 and 18:0). The diol profile is similar to profiles reported by other workers (e.g. Deprez *et al.*, 1990; Kayama *et al.*, 1971).

There has been some suggestion that diacylgylceryl ethers are important in the treatment of haematopoiesis and radiation sickness (Blomstrand and Ahrens, 1959). The abundance of these lipids from diverse sources including deep-sea sharks, many marine oils and various mammalian species may provide future stimulation for the pharmaceutical, health product and related industries to prepare a range of products (Blomstrand and Ahrens, 1959; Nichols *et al.*, 1993). At this time, DAGEs are not widely used commercially, although there has been suggestion of their use as non-fattening lipids as a replacement for triacylglycerols (Mangold, 1983).

CONCLUSION

The large bycatch of shark (both relative and absolute amounts) in the deep-sea fishing industry in Australian waters provides a useful source for possible additional income to fishermen. In addition, most species analysed in this study are predominantly caught in large quantities during commercial trawling in Australian waters. Production of liver oil will enable the fishing industry to further exploit bycatches of deep-sea sharks for the rich sources of squalene and diacylglyceryl ethers. We have shown that the sharks analysed here have markedly different proportions of individual lipids in their liver oil. As the final value of the oil is based on levels of individual components, it may be necessary to separate the different shark species at the point of harvest where possible in order to increase processing efficiency.

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Table Headings and Legends

Header

Table 1. Lipid composition of liver oils from deep-sea sharks collected in southern Australian waters.

Footer

HC, Hydrocarbon (predominantly squalene); DAGE, Diacylglyceryl ethers; TAG, Triacylglycerols; FFA, Free Fatty Acids; PL, Polar Lipids.

Header

Table 2. Total fatty acid composition of liver oils from southern Australian deep-sea sharks[A].

Footer

[A]All values are expressed as a percentage of the total fatty acids unless otherwise stated. GC results are subject to an error of ±5%.

- not detected in sample

Table 1. Lipid composition of liver oils from deep-sea sharks collected in southern Australian waters

Shark species	Common Name	Percentage Composition						
		HC	DAGE	TAG	FFA	PL	OTHER	TOTAL
Somniosus pacificus	Pacific sleeper shark	-	49.5	49.1	-	1.4	-	100
Centroscymnus plunketi	Plunket shark	0.9	76.6	22.5	-	-	-	100
Etmopterus granulosus (2)	Lantern shark	50.3	32.1	9.3	8.3	-	-	100
Etmopterus granulosus (1)	Lantern shark	60.5	29.8	6.8	-	0.7	2.2	100
Deania calcea	Platypus shark	69.6	19.6	10.8	-	-	-	100
Centroscymnus crepidater	Long-nose velvet shark	73.0	20.0	5.0	-	0.7	1.3	100
Centrophorus scalpratus	Endeavour shark	81.6	9.9	8.5	-	-	-	100

HC = Hydrocarbon (predominantly squalene), DAGE = Diacylglyceryl ethers, TAG = Triacylglycerols
FFA = Free fatty acids, PL = Polar lipids

able 2. Total fatty acid composition of liver oils from southern Australian deep-sea sharks[A].

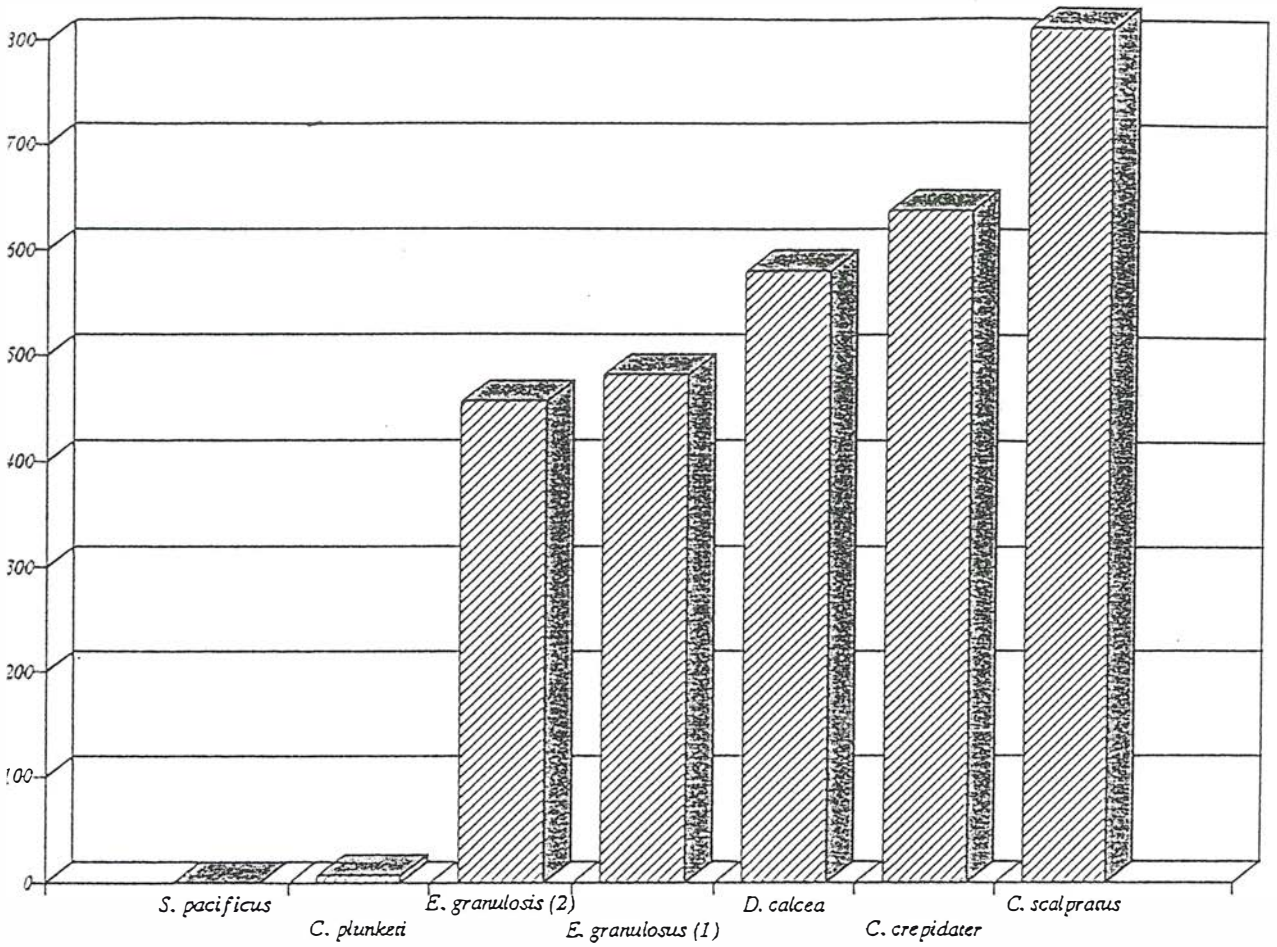
Fatty Acid	Percentage Composition						
	S. pacificus	*C. plunketi*	*E. granulosis (2)*	*E. granulosus (1)*	*D. calcea*	*C. crepidater*	*C. scalpratus*
14:0	0.5	0.4	0.9	0.9	0.9	0.8	1.5
15:0	0.1	0.2	0.3	0.3	0.3	0.2	0.4
16:2(n-7)	0.1	0.1	0.1	0.1	0.0	0.4	0.0
16:1(n-9)c	0.6	0.2	0.6	0.5	0.3	0.4	0.3
16:1(n-7)c	3.6	2.8	2.2	2.0	3.2	2.4	3.9
16:0	9.4	9.1	11.4	12.3	21.3	12.7	19.8
i17:1	0.3	0.5	0.6	0.6	0.4	0.7	0.5
i17:0	0.3	0.3	0.3	0.3	0.3	0.2	0.1
al 7:0	0.7	0.7	0.6	0.5	0.8	0.6	0.6
17:0	0.2	0.1	0.2	0.2	0.3	0.1	0.3
18:3(n-3)	-	-	-	-	-	-	0.5
18:2(n-6)	1.4	0.8	0.4	0.4	0.8	0.7	1.0
18:1(n-9)c	48.8	36.8	23.6	22.9	30.3	30.2	37.6
18:1(n-7)c	7.1	1.9	2.8	2.6	2.3	2.6	3.0
18:1(n-5)c	0.3	0.1	0.1	0.1	0.1	0.1	0.1
18:0	2.0	1.3	1.8	1.7	2.5	1.4	3.1
i19:0	0.3	0.3	0.3	0.2	0.2	0.2	0.2
a19:0	0.4	0.3	0.3	0.3	0.2	0.2	0.2
19:0	0.1	-	0.0	0.1	0.1	-	0.1
20:5(n-3)	0.7	-	0.1	-	0.8	-	1.0
20:4(n-6)	0.7	-	0.1	-	0.4	-	0.5
20:2(n-6)	0.6	0.3	0.2	0.1	0.3	0.1	0.2
20:1(n-9)t	-	-	2.8	1.9	4.0	1.4	1.5
20:1(n-9)c	2.0	21.0	18.6	16.8	10.1	16.5	7.7
20:1(n-7)c	0.9	0.7	0.5	0.4	0.5	0.4	0.4
20:0	0.1	0.2	0.2	0.3	0.3	0.2	0.2
21:1	-	0.2	0.1	0.2	0.1	0.2	-
22:5(n-6)	0.2	0.1	-	-	0.2	-	0.2
22:6(n-3)	7.0	1.2	1.4	0.7	4.2	0.6	5.9
22:4(n-6)	0.5	0.1	0.0	-	0.1	-	0.2
22:5(n-3)	2.2	0.2	0.2	-	0.4	-	1.0
22:1(n-11)c	3.5	12.7	20.2	22.9	8.9	15.8	3.6
22:1(n-7)c	2.3	2.9	2.7	3.0	1.5	3.0	1.4
22:1	0.2	0.2	0.4	0.4	0.2	0.3	0.2
22:0	0.1	0.1	0.2	0.5	0.2	0.2	0.2
24:1	0.6	1.1	1.5	1.9	0.6	2.3	0.4
24:1(n-9)c	2.0	3.0	3.8	4.5	2.7	4.8	2.1
Other	0.2	0.1	0.3	0.5	-	0.4	0.1
Total	100	100	100	100	100	100	100
Σ Saturates	12.5	11.5	26.1	16.2	26.1	15.7	25.6
Σ Monounsaturates	72.0	83.6	64.8	80.1	64.8	80.2	62.2
Σ Polyunsaturates	13.3	2.6	7.2	1.3	7.2	1.4	10.6
Total Fatty Acid (mg/g)	340	575	365	250	235	170	95

All values are expressed as a percentage of the total fatty acids unless otherwise stated. GC results are subject to an error of ±5%.
ot detected

Figure Legends

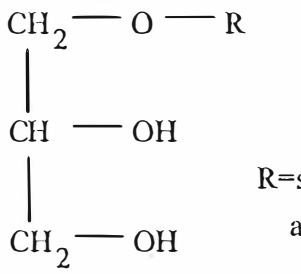
Figure 1. Absolute amounts of squalene in the liver oil from six species of deep-sea sharks caught in southern Australian waters.

Figure 2. Capillary gas chromatogram of glyceryl ether diols in neutral lipid fractions of liver oils from six species of deep-sea sharks caught in southern Australian sharks. A, unidentified; B, 14:0 diol; C, unidentified; D, unidentified; E, 16:1(n-7)c diol; F, 16:0 diol; G, 18:1(n-9)c diol; H, 18:1(n-7)c; I, 18:0 diol; J, 20:1(n-9) diol; K, 20:1(n-7) diol.



Species of shark

General structure of glyceryl ether diols.



R=saturated or monounsaturated alkyl chain

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Title:

Nutritional analysis of the flesh and oil of the Tasmanian mutton bird *Puffinus tenuirostris*: useful sources of omega-3 fatty acids

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Abstract

Objective: To investigate the chemical composition of the cooked skinless flesh and the proventricular (stomach) oil of the mutton bird.
Results: The flesh has high levels of vitamin A, iron, zinc, calcium, iodine and selenium. Its fat (18.7% by weight) has a high proportion of omega-3 polyunsaturated fatty acids (9.3% of total fatty acids). The oil is similarly rich in omega-3 fatty acids (24.9% of total fatty acids).
Conclusions: Regular consumption of the flesh may assist in management, and primary prevention, of atherogenic and thrombogenic conditions. The oil may have therapeutic usefulness for atherosclerotic and thrombotic conditions (and has definite potential in the mariculture industry).

Introduction

Over the last decade, there has been an upsurge in interest in the nutritional properties of traditional Aboriginal foods [1]. However, most analyses relate to northern Australia species. Little attention has been given to those of the southern region, including Tasmania.

One species important for Tasmanian Aborigines before and after European contact is the mutton bird [2,3], called 'yolla' in the local languages [4]. Aboriginal communities are expanding an industry based on this species. This present study investigated the nutritional value of two products derived from the mutton bird: its flesh and its oil. (The oil, whose physiological function for the bird is uncertain [5], is obtained from the proventriculus (stomach) by inverting and squeezing the bird; the body, as sold for eating, does not contain any oil.).

Materials and methods

Analysis of flesh.

Six skinless birds (without heads, wings, legs or entrails), from the Aboriginal enterprise Yolla Products were delivered frozen to the SA branch of the Australian Government Analytical Laboratories.

For analysis, they were thawed, browned in their own fat and braised in tap water until the flesh was tender (about 2 h). All edible parts, including flesh and visible fat, were homogenised to form a composite sample. Samples were refrigerated until analysis, with moisture and vitamin assays begun within 24 h to minimise losses [6]. All methods are used routinely in these laboratories for analysis of other Australian foods (e.g., in ref 7).

Energy and macronutrient analyses. Moisture was determined by drying at 102°C, ash by ignition at 550°C, fat by Soxhlet extraction, and total protein by a Kjeldahl procedure [8]. Carbohydrate was calculated by difference, and energy using the factors fat 37 kJ/g, protein 17 kJ/g and carbohydrate 16 kJ/g [9].

Minerals. Flame atomic absorption spectrophotometry was used to assay sodium and potassium (after wet ashing in nitric acid), calcium, magnesium and zinc (after dry ashing at 400°C), iron (after wet ashing in nitric and perchloric acids) and selenium (ashed as for iron, and then converted to selenium hydride). Iodine was analysed by inductively-coupled plasma mass spectrometry (MS), in the NSW Regional Laboratory of AGAL.

Vitamins. Thiamin was extracted by acid and enzyme digestion, separated by high pressure liquid chromatography (HPLC), and assayed fluorometrically as thiochrome after post-column oxidation by potassium ferricyanide [10]. Niacin was determined colourimetrically by the Konig reaction with cyanogen bromide after alkaline extraction - a procedure which detects both nicotinic acid and nicotinamide. Retinol and beta-carotene were extracted from the sample under subdued light using petroleum ether, after preliminary hydrolysis with alcoholic KOH. The extracts were taken to near dryness with nitrogen gas, re-dissolved in methanol and subjected to reverse phase HPLC (C18 Nova Pak column, Waters 490 u.v./visible detector). For retinol, the mobile phase was methanol: water (95:5), with the detector set at 325 nm; for beta-carotene, methanol: tetrahydrofuran (90:10) and 450 nm were used.

Cholesterol. In the petroleum ether extract used for the retinol assay, cholesterol was derivatised using acetic anhydride, and determined by capillary gas chromatography (GC) on a 12m non-polar bonded phase capillary column (SGE, 12QC2/BPI).

Fatty acid profile. Lipids - including triglycerides, phospholipids, and possibly waxes - were extracted using chloroform: methanol (1:2) [11], and trans-esterified to methyl esters using sodium methoxide in methanol. After re-extraction into hexane, the methyl esters were determined on a 25m polar bonded phase capillary column (SGE, 25Q2/BPX-70). Individual fatty acids were identified by comparing retention time data with data for a range of authentic standards. Identifications were independently confirmed by GC-MS.

Analysis of oil

The opaque, viscous orange- red oil was supplied by Yolla Products, and analysed at CSIRO Marine Laboratories, Hobart. Lipid class abundances were measured by Iatroscan MK III TH10 TLC-FID analysis [12]. Fatty acid methyl esters, formed by trans-esterification of the lipids (HCl /chloroform / methanol; 1:1:10 v/v/v, 100^{o}C, 2 hr) were analysed by GC, with a cross-linked methyl silicone capillary column, FID and a split/splitless injector, using hydrogen as carrier gas [13].

Results

The composition of the cooked skinless flesh is presented in Table 1, with the fatty acid profile of the flesh lipids in Table 2.

The proventricular oil comprised 32% triglycerides and 64% waxes, with small amounts of free fatty acid, polar lipid, sterol and alcohol. Table 3 lists the fatty acid profile of the oil. (The oil also contains various alcohols, derived from the waxes: 61.5% were saturated, mainly 14:0 10.7% and 16:0 45.7%; 38.5% monounsaturated, mainly 20:1 *w9cis* 8.6% and 22:1 *w11cis* 6.5%.)

Discussion

Compared with similarly-prepared foods that it might replace in an Australian diet (e.g., lean beef, skinless chicken, or a steamed fish), mutton bird flesh is rich in vitamin A, iron, calcium and zinc [7]. While comparisons with other foods are not available, its iodine and selenium levels are such that 100 g would supply 92% of an adult male's recommended daily intake (RDI) for selenium, and 180% of his iodine RDI [14]. A minor disadvantage is the Na:K (molar) ratio of 1.3, which is a little higher than the desirable 'value of < 1.0 [15].

The fat content of the flesh is high (18.7%), but its fatty acid profile has excellent cardiovascular properties. Its PMS (polyunsaturated: monounsaturated: saturated) ratio is 0.47: 2.31: 1.00, more favourable than most other flesh foods [7]. The polyunsaturated fat is mainly of the omega-3 type (normally found only to any useful extent in seafood) which retards thrombogenesis, and lowers blood pressure [16]. Consumption of a mere 100g per week of mutton bird flesh would double a typical Australian's intake of omega-3 fatty acids [17]. Ulbricht & Southgate's [18] indices, which weight fatty acids according to cardiovascular effects, are: for atherogenicity, 0.38 (similar to polyunsaturated margarine or fish, and more favourable than beef or chicken), and for thrombogenicity, 0.33 (much more favourable than beef, chicken or polyunsaturated margarine, and approaching that of fish).

The oil, although it has a different anatomical origin, has a PMS ratio (2.92: 6.10: 1.00), atherogenicity index (0.21), and thrombogenicity index (0.08) that are all substantially more favourable than the flesh itself. Consumption of 1 g oil per day would double a typical Australian's intake of omega-3 fatty acids [17].

Thus, the lean flesh offers substantial nutritional benefits. In particular, because of its fat composition, regular consumption would make it a useful dietary adjunct for management of atherosclerotic or thrombotic conditions, and may contribute to primary prevention of these conditions. Arguments adduced for increased fish consumption apply equally for mutton bird [17]. However, our nutritional analysis and discussion are based on skinless birds, cooked without added fat, and not soaked in brine. Different preparation procedures may significantly reduce nutritional desirability.

The oil may be suitable as a dietary supplement for those with atherosclerotic or thrombotic conditions, perhaps administered in capsule form. However, bioavailability needs further investigation: for triglyceride fatty acids it should be high, but for wax fatty acids it is uncertain.

The oil also has potential non-clinical usefulness in the mariculture industry, because of its high content of polyunsaturated fatty acids and wax esters, and because its rich red colour suggests a high content of valuable astaxanthin or related pigments.

Is increased harvesting of the birds consistent with sustainable yield? The industry is already subject to conservation and public health control by the state government. Within the framework of these controls, a substantial increase in mutton bird harvesting would not threaten the long-term survival of the species [19, 20]. Increased harvesting would improve the economic outlook for several remote Aboriginal communities.

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Table 1. Composition (per 100 g edible portion) of cooked skinless mutton bird flesh.

Energy	
kJ	1120
Macronutrients	
protein (g)	23.9
fat (g)	18.7
carbohydrate (g)	1.1
Vitamins	
retinol (mg)	0.45
beta-carotene (mg)	0
pre-formed niacin (mg)	3.2
thiamin (mg)	0.14
Minerals	
calcium (mg)	46
iodine (mg)	0.27
iron (mg)	4.5
magnesium (mg)	33
potassium (mg)	190
selenium (mg)	0.078
sodium (mg)	150
zinc (mg)	2.5
Lipids	
cholesterol (mg)	185

Table 2. Fatty acid profile of skinless mutton bird flesh lipid (as % of total fatty acids)[1]

Saturated	Monounsaturated	Polyunsaturated omega-3	omega-6
14:0 2.5%	14:1 0.2%		
15:0 0.3%			
16:0 18.1%	16:1 8.0%		
17:0 0.2%			
18:0 5.1%	18:1 36.7%		18:2 2.3%
20:0 0.3%	20:1 8.8%	20:3 0.2%	20:2 0.2%
		20:5 3.3%	20:4 0.8%
	22:1 6.6%	22:5 0.6%	
		22:6 5.2%	
	24.1 0.6%		
Total 26.5%	Total 60.9%	Total 9.3%	Total 3.3%

[1] Individual fatty acids comprising <0.2% of total fatty acids not shown, but included in totals. In accordance with standard convention, fatty acids are denoted as X:Y, where X is the number of carbon atoms and Y the number of double bonds.

Table 3. Fatty acid profile of mutton bird oil (as % of total fatty acids) [1]

Saturated	Monounsaturated[2]	Polyunsaturated [3] omega-3	omega-6
14:0 3.3%	14:1 0.2%		
16:0 5.6%	16:1 15.7%		
17:0 0.2%	17:1 0.7%		
18:0 0.8%	18:1 36.3%	18:3 0.7%	18:2 2.2%
		18:4 2.3%	
	20:1 3.3%	20:4 0.7%	20:2 0.2%
		20:5 12.5%	20:3 0.3%
			20:4 0.5%
	22:1 3.8%	22:5 0.5%	
		22:6 7.8%	
	24.1 0.8%		
Total 10.0%	Total 60.9%	Total 24.9%	Total 2.7%

[1] See note 1, Table 2.

[2] 14:1, all *w5cis*; 16:1, *w7cis* 15.5%, *w5cis* 0.2%; 17:1, *w8cis*; 18:1, *w9cis* 30.9%, *w7cis* 4.8%, *w5cis* 0.6%; 20:1, *w9cis* 2.6%, undetermined (but not *w7cis*) 0.7%; 22:1, *w11cis* 2.9%, *w11trans* 0.9%; 24:1, double bond position undetermined.

[3] Other polyunsaturated fatty acids: 16:4 *w1*, 0.7%; 16:3 *w4*, 1.0%.

Note to editor: Throughout this Table, *w* represents Greek lower-case omega.